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FURTHER OBSERVATIONS ON THE TRANSMISSION AND DEVELOPMENT OF LEUCOCYTOZOON SIMONDI[1]

BY A. M. FALLIS, R. C. ANDERSON, AND G. F. BENNETT

Abstract

Observations are presented on the incidence of *Leucocytozoon simondi* in ducklings during several summer seasons. Species of black flies of the subgenus *Eusimulium* (*Simulium croxtoni* and *Simulium euryadminiculum*) and *Simulium rugglesi* feed on ducks and have been shown to be suitable hosts for *L. simondi*. The former species appear to be the important vectors of the parasite in the early part of the black fly season and the latter species in late June and July. Development of the parasite in *S. rugglesi* has been followed. Oöcysts occur in, and on, the stomach wall, and sporozoites are found in the salivary glands. The small oöcysts contain relatively few sporozoites. The data suggest, but do not prove conclusively, that sporogony, although variable, can be completed in three or four days. Specimens of *S. rugglesi* contained viable sporozoites 18 days after the flies had their final blood meal containing gametocytes. The splenomegaly, changes in the composition of the blood, size of parasites, and presence of asexual stages in the tissues, were observed in infected ducks in relation to the time of infection and as a result an hypothesis is presented regarding the asexual cycle.

A previous report (7) included information on the development of *Leucocytozoon simondi* in *Simulium venustum* Say although all stages of the parasite were not found. Further studies have indicated that *S. rugglesi* Nicholson and Mickel and species of the subgenus *Eusimulium* are more important vectors than *S. venustum* and that previous conclusions about transmission and development of the parasites should be modified. Development in *S. rugglesi* has been followed and is reported herein together with observations on the transmission of the parasite throughout several summer seasons and on the prevalence of different species of black flies that are suitable intermediate hosts. Additional data are presented also on the development of the parasite within ducks.

Materials and Methods

The work was done in the same locality as that reported previously (7) using White Pekin ducklings that were one to three weeks of age at the beginning of an experiment. They were exposed in open-top pens of 1 in. wire mesh or in cages covered with copper mesh wire having openings of either 0.035 in. or 0.0107 in. The latter type was flyproof and was used also for

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holding non-infected ducks in the animal house. Blood smears were made from the ducks at various intervals beginning usually five days after exposure to infection. Samples of the black flies that were feeding naturally on ducks were obtained by placing ducks, exposed for various intervals, inside cages covered with fine nylon mesh and, after 20 min., collecting the flies that had come off the ducks. Infected black flies were comminuted in blood or physiological saline in a tissue grinder and the resulting suspensions were injected intraperitoneally into parasite-free ducks. Flies that were to be sectioned were fixed in Bles fluid for 48 hr. and then preserved in 70% alcohol. Heparinized blood was transferred from infected to parasite-free birds.

Natural Transmission and Species of Black Flies Feeding on Ducks

A measure of the natural transmission of *Leucocytozoon* to ducks in one locality was obtained during the past six years by placing several hundred ducklings in the open in Algonquin Park at intervals that extended each year from the middle, or latter part, of May, until mid-August or September and noting when they became infected. The types of bloodsucking diptera in the area were noted also and, throughout the summer of 1955, black flies which fed naturally on ducks were collected and their suitability as intermediate hosts for *L. simondi* was tested. The possible role of *Culicoides* spp. as vectors was tested also. The pattern of natural transmission during each of the years 1950–1954 was essentially the same and will be summarized only. Transmission during 1955, especially in relation to the species of black flies known to feed on ducks, was followed closely and the observations are presented in more detail.

Ducks exposed during the last week of May in 1950 and 1951 became infected and a high rate of transmission to other ducks occurred throughout June and July. Transmission during May was not observed in 1952–1954 but it occurred during June and July. More than 200 ducks, exposed continuously during these two months, became infected and more than 50% of them died, presumably from the infection. Parasitaemias were detected in many of the ducks six days after exposure, although in some instances the prepatent period was shorter. Infection developed in some of the ducks that were exposed during August, but the time from exposure to detection of parasitaemia was 8 to 13 days indicating a lower rate of transmission than in June and July. Transmission throughout June is in contrast to the observations of O'Roke (12) and Chernin (2) who reported a high rate of transmission in July in northern Michigan.

No evidence of transmission was obtained in any year prior to the appearance of black flies. *Prosimulium hirtipes* (Fries) usually is the first to appear in Algonquin Park followed by *Simulium venustum* Say and the other species listed by Davies (5). The high rate of transmission in June and early July coincided with an abundance of *S. venustum*, which was regarded for several years (7, 12) as an important vector. This species was relatively scarce in July however, and *S. parnassum* Malloch, although frequently

present, did not seem sufficiently abundant to account for the high incidence of *Leucocytozoon* that was obtained. The scarcity of these black flies was noticeable especially during July 1953 and 1954 and at the same time *Culicoides* spp. were abundant. It was decided, therefore, to test whether the latter would act as vectors.

In the first experiment beginning June 19 ducks were exposed for 11 days, three in an open pen, four in a cage having 0.035 in. openings, and four in a cage having 0.0107 in. openings. Sand flies, but presumably not black flies, could enter the former cage but none could go through the latter. Parasites were seen in the peripheral blood of the three ducks in the open pen six days after exposure. Infection was not observed in any of those in the other two cages.

The experiment was repeated beginning June 25 using two ducks in the open pen, two in the cage with coarse mesh, and four in the cage with fine mesh. Infection was apparent six days after exposure in the ducks in the open pen but never appeared in the ducks in the other two cages.

In a third experiment six ducks were kept from July 10 to 30 in the coarse mesh cage but none of them became infected. During this same period 27 ducks were exposed in the open pen and all had parasites in their blood 6–10 days after exposure. Sand flies were abundant during this entire time.

Many sand flies were collected during this same period and comminuted in serum and Ringer's solution. These suspensions, containing 220, 320, 60, 280, 100, and 375 sand flies respectively, were injected either intravenously or intraperitoneally into six parasite-free birds. No infections resulted. Four ducks were injected with suspensions of 15, 2, 2, and 10 sand flies that had fed on infected ducks 3, 4, 5, and 7 days previously. No infections were produced by these injections. The results of these observations and experiments indicate that species of *Culicoides* in this locality are unsuitable hosts for *L. simondi*.

Ducks were kept under close observation during that part of July 1954 when black flies seemed scarce but when sand flies were abundant. One species of black fly, *S. rugglesi*, was observed crawling about and feeding on ducks when few black flies were in the vicinity and when none were found feeding on man. Several gorged specimens of these black flies* were captured as they came off an infected duck. Although specimens of *S. rugglesi* had been taken off a duck as far back as 1950 (during which year the description by Nicholson and Mickel (11) appeared) by Davies (6) they were not identified at the time and the suitability of this species as a host for *L. simondi* was not tested until 1954.

A few specimens of *S. rugglesi* that were taken from infected ducks in 1954 were maintained in captivity. Two of these flies were ground with serum in a tissue grinder 92 hr. after they had fed on the infected duck and the resulting

*Some of these flies were identified as S. rugglesi by Dr. A. Stone, U.S. Bureau of Entomology, Mr. G. E. Shewell, Division of Entomology, Science Service, Canada, and Prof. D. M. Davies of McMaster University, to each of whom we are grateful for their assistance.

suspension was injected intraperitoneally into a parasite-free duck. Three additional flies were kept in captivity for six and one-half days, then comminuted in serum, and the suspension was injected into a second parasite-free duck. Heavy infections developed in each of these ducks indicating that *S. rugglesi* is a suitable host for this parasite.

It was decided, therefore, to follow more closely in 1955, the natural transmission of *L. simondi* to determine which species of black flies feed naturally on ducks and which of these species are hosts for the parasite. Black flies that fed on infected ducks at different times throughout the summer were collected as reported by Anderson (1). It was noticed, in some cases, that 15–20 min. elapsed from the time the flies landed on the duck until they fed and flew off again. Some of these flies, following identification, were sectioned, others were dissected to look for stages of the parasite, and others were injected into parasite-free ducks to determine whether the flies were carrying sporozoites. The species composition of the samples of flies collected in this way varied throughout the season as reported by Anderson (1). Gorged specimens of *Simulium croxtoni* Nicholson and Mickel, *S. euryadminiculum* Davies, and *S. latipes* (Meigen) of the subgenus *Eusimulium* were taken from May 21 to June 15 although only a few were taken after June 6. Gorged specimens of *Simulium rugglesi* were taken from May 26 to July 10; this species was most common in the latter part of June. These observations are proof for the prediction of Shewell (14) that species of black flies with a basal tooth on the tarsal claw are likely to be ornithophilic. The scarcity of black flies after July 10 in 1955 is in contrast to the observations during any of the previous five years. In view of this and the fact that flies were recovered from ducks earlier in the season, it is of interest to note the incidence of infection in ducks that were exposed periodically throughout the summer of 1955 (Table I). Infection was detected in several ducks on May 28. These were

TABLE I

NATURAL INFECTIONS WITH *L. simondi* IN 1955 IN DUCKS IN EXPERIMENTAL AREA IN ALGONQUIN PARK

No. ducks exposed	Date exposed	Dates parasites detected
5	May 9	May 28
2	May 17	May 28
4	May 18	May 28
5	May 29	June 4
10	June 4	June 10
5	June 14	June 21–28
10	June 26	July 7– 9
7	July 2	July 9–10
8	July 9	July 16–19
5	July 17	(1)* Aug. 7
4	July 25	(1)* Aug. 4
		(1)* Aug. 27
4	Aug. 2	Negative Sept. 1

**Number of ducks infected.*

most probably infected on May 21 or May 22 on which days *Eusimulium* spp. were known to be feeding on ducks as shown by Anderson (1). Transmission continued throughout June and up to July 9 during which time 69 ducks were exposed. All of them became infected with *Leucocytozoon* and many of them died, presumably from the infection. *Leucocytozoon* was detected in only 3 of the 13 ducks that were exposed after July 9. These observations, considered along with those on the black flies that were known to feed on ducks on different days during the summer (1), lead to the belief that *L. simondi* is transmitted to ducks in Algonquin Park by species of the subgenus *Eusimulium* during the first part of the season and by *S. rugglesi* in the latter part.

Although *S. rugglesi* had been shown in 1954 to be a suitable host it seemed desirable to obtain further proof of this fact. This was done (*a*) by examining dissected and sectioned specimens of flies that had fed on infected ducks and looking for developing stages of the parasite and (*b*) by comminuting flies at various intervals after they had fed on infected ducks and injecting the suspensions into parasite-free ducks. The results of a series of such injections are reported in Table II. They confirm the previous observations that *S. rugglesi* is a suitable host for *L. simondi*. Moreover flies may remain infective for at least 18 days after ingesting gametocytes. Some of the flies must have been infected prior to their last blood meal as infection was produced in one duck that received flies that fed less than four hours previously.

TABLE II

SPECIMENS OF *S. rugglesi* THAT WERE INJECTED INTO DUCKS AT INTERVALS AFTER THEY HAD FED ON INFECTED DUCKS

Date of injection	No. of flies	Days from feeding to injection	Parasites noted
31/5	3	3 and 4	−
3/6	2	1	−
9/6	2	7	−
11/6	10	18 hr.	−
11/6	1	9	−
11/6	2	$3\frac{3}{4}$	−
11/6	2	$4\frac{3}{4}$	−
14/6	2	4	−
15/6	1	5	−
16/6	2	6	−
15/6	12	1–4 hr.	+
17/6	4	2	−
18/6	4	3	−
19/6	5	4	+
20/6	4	5	+
21/6	4	6	+
22/6	4	7	+
23/6	4	8	+
24/6	4	9	+
2/7	4	13	+
7/7	3	18	+
11/7	3	7–9	+

TABLE III

BLACK FLIES OF THE SUBGENUS *Eusimulium* THAT WERE INJECTED INTO DUCKS AT INTERVALS AFTER THEY HAD FED ON INFECTED DUCKS

Date of injection	Species of fly	No. of flies	Days from feeding to injection	Parasites noted
25/5	*S. croxtoni*	4	3	−
26/5	*S. croxtoni*	4	5	−
26/5	*S. croxtoni*	3	4	+
27/5	*S. croxtoni* *S. euryadminiculum*	3 1	6	+
31/5	*S. latipes*	2	4	−
31/5	*S. euryadminiculum*	2	3 and 4	+
31/5	*S. croxtoni*	6	3 and 4	+
3/6	*S. croxtoni, S. latipes, S. euryadminiculum*	11	1	+

It seems unlikely that failure to produce infection with the flies that were injected on May 31 and in early June was due to lack of time for sporogony unless more than seven to nine days is required for its completion. The series of injections from June 17 to July 7 inclusive comprise a single experiment in which the flies had fed, on June 15, on the same heavily infected ducks. Twelve of these flies were injected, within four hours after feeding, into a duck which, as a result, developed infection. Consequently some of these flies must have been infected by a previous blood meal or they contained asexual parasites capable of initiating an infection. Otherwise the infections resulting from the other injections suggest, but do not prove, that sporogony is completed in four days.

The suitability of *S. croxtoni*, *S. euryadminiculum*, and *S. latipes* as intermediate hosts was tested although fewer ducks were injected than with *S. rugglesi*. Moreover, relatively low parasitaemias were present in the ducks on which specimens of these species of *Eusimulium* were known to have fed. Nevertheless it is apparent (Table III) that *S. croxtoni* and *S. euryadminiculum* are satisfactory hosts. The results suggest also that sporogony may be completed in four days although the possibility that some flies were infected prior to their last blood meal should not be excluded as infection resulted in one instance from the injection of flies that ingested gametocytes the previous day.

Sporogony in *S. rugglesi*

Dissections of specimens of *S. venustum*,* reported previously (7), revealed structures that were regarded as sporozoites and developing oöcysts. Mature oöcysts were not located although these had been reported by O'Roke (12) in

**The possibility that some of these were S. rugglesi cannot be excluded completely.*

flies that were considered to be *S. venustum* but which may have been *S. rugglesi* (14).† Sporozoites of *L. smithi* in the salivary glands of *Simulium nigroparvum* (Twinn) = (*S. jenningsi* Mall.) had been found by Johnson *et al.* (10). Further knowledge of sporogony seemed desirable especially in *S. rugglesi* which was known to feed readily on ducks and which was known to be a satisfactory host. Specimens of *S. rugglesi* which had fed on infected ducks were maintained for this purpose in captivity using Anderson's method (1). Some of these flies were fixed, for sectioning, at various intervals after their blood meals, others were dissected in physiological saline and the resulting wet preparations were examined or the preparations were dried and stained with Giemsa's stain before examination.

Exflagellation and early development is like that described (7, 12). Variation was noticeable in the rate of development within a single fly as well as in different flies. Some oökinetes persisted in the stomachs of some flies 72–96 hr. after the ingestion of parasites. Although the transformation of an oökinete into an oöcyst was not followed completely it was noticed in some flies, dissected 48 hr. after they had ingested gametocytes, that the chromatin in some oökinetes was divided into a number of separate entities. In our previous dissections (7) structures that were thought to be developing oöcysts were found in the stomachs of some flies about 42 hr. after they ingested gametocytes. Since then similar structures have been seen in the stomach contents as well as in the stomach wall of specimens, identified as *S. venustum*, that were sectioned 36 hr. after they had ingested gametocytes (Fig. 1). Similar round structures were observed in stained smears of the stomach content and in sections of *S. rugglesi* made 24–48 hr. after the last blood meal of the fly. Oöcysts were found within the stomach wall as well as bulging into the haemocoele of *S. rugglesi* 48–72 hr. after the fly had fed on an infected duck (Figs. 2, 3, 4, 5). The position of some of these oöcysts within the wall suggests that the parasite may be intracellular thus resembling *Plasmodium circumflexum* as described by Reichenow (13). Development of some oöcysts undoubtedly takes place more slowly than this as some were observed three and four days after the blood meal in flies that fed at the same time and on the same ducks, as those just mentioned.

Twenty-five oöcysts containing active sporozoites were measured in a preparation in which the stomach of an infected fly was mounted in saline beneath a cover glass. Their average diameter was 11.4 μ. Each oöcyst contained relatively few sporozoites. Sporozoites resembling those illustrated previously were found in the salivary glands of flies that were sectioned, and in others that were dissected, three and four days after the ingestion of parasites. Sporozoites reach the haemocoele presumably from the rupture of oöcysts into it although sporozoites were found among the contents of the stomach of a specimen of *S. rugglesi* that was dissected 72 hr. after the fly

†*Dr. O'Roke has kindly permitted me to quote from a personal communication in which he says in part "I think the "white stockinged" black flies with which I worked at the University of Michigan Biological Station in the early thirties may well have been Simulium rugglesi instead of S. venustum".*

had fed. Possibly in this instance the sporozoites were from oöcysts that ruptured when the stomach was dissected open, although it could be a normal occurrence if sporozoites themselves can penetrate the stomach wall or otherwise reach the salivary glands.

The regularity with which oöcysts were found in the stomach wall of black flies two to four days after the flies ingested many gametocytes suggests that sporogony can be completed in this time. This assumes, of course, that the observed oöcysts developed from the parasites ingested with the last blood meal. Observations suggest that the rate of development of oökinetes into oöcysts and the time of movement through the stomach wall may depend on the rate of formation of a peritrophic membrane and on the rate of digestion of the meal. Oökinetes in contact with the stomach wall before the membrane is formed may be responsible for the oöcysts seen at 48–72 hr. while those remaining in the stomach contents may not reach the wall to complete their development until they can escape through a break in the membrane. This would account for the presence of some oökinetes in the stomachs of some flies 72 or more hours after the ingestion of gametocytes. Variation in the rate of development and survival of the sporozoites for many days in the salivary glands suggests that a fly, once infected, remains so throughout its life. The presence of oöcysts on the outer wall of the stomach indicates an error in a previous opinion (7) which implied that oöcyst formation occurred within the stomach. Although this possibility should not be rejected completely it is now clear that some, and perhaps all, oöcysts do appear on the outer wall of the stomach and that sporozoites are present in the salivary glands.

Further Observations Relating to the Asexual Development

Earlier studies (7) of the asexual development revealed at least two types of schizonts, one of which (megaloschizont) was found in a variety of tissues. It was clear also that parasites capable of initiating an asexual cycle were present, at times, in the blood. Fallis *et al.* (7) concluded that an asexual cycle was completed in fewer than five days. Studies by Cowan (3) have suggested that less time may be required. It seemed desirable therefore to investigate further the rate of asexual development and the sequence of th various stages. This was done by (*a*) following the size of parasites on different days of patency and relating this to the length of the prepatent period, (*b*) artificial transmission of the parasites by transfusions of blood removed from infected ducks at different times after they were infected, (*c*) noting the changes in the composition of the blood of ducks on successive days following their infection, (*d*) examining sections of different tissues removed from ducks at various times following their exposure to infection.

Growth of Gametocytes

The presence of parasites in the peripheral circulation of ducks five to six days after exposure indicates the completion of at least one asexual cycle. The parasites seen at this time are usually small although in a few instances

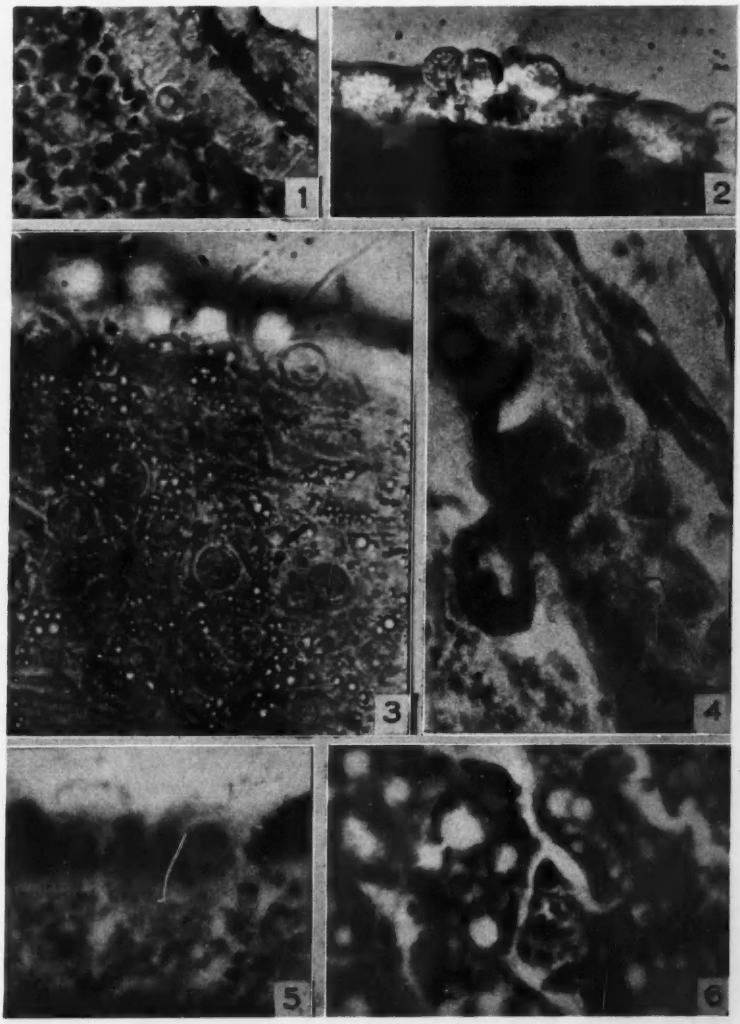

FIG. 1. Section through stomach of *S. venustum*, fixed 36 hr. after ingestion of gametocytes, showing structure regarded as developing oöcyst in stomach wall and another among stomach contents. *ca.* 600 ×. FIG. 2. Fresh preparation of stomach of *S. rugglesi* mounted in saline. Oöcysts containing sporozoites are apparent on outer wall of the stomach. The fly had ingested gametocytes 51 hr. prior to dissection but the oöcysts in the illustration could be the result of a previous feeding. *ca.* 600 ×. FIG. 3. Same preparation as Fig. 2 but focus is on a lower level to show oöcysts, some with developing sporozoites, lying below the outer surface of the stomach. FIG. 4. Section of *S. rugglesi* that was fixed 72 hr. after last ingestion of gametocytes. Developing oöcysts are seen at different levels of stomach wall, and some appear intracellular. *ca.* 600 ×. FIG. 5. Section of *S. rugglesi* that was fixed 50 hr. after last ingestion of gametocytes. Developing oöcyst appears to be intracellular in stomach wall. *ca.* 600 ×. FIG. 6. Section of liver of duck three to five days after exposure to infection with *L. simondi*. Structure in Kupffer cell thought to be a schizont. (Courtesy R. C. Ritchie.) 600 ×.

we have observed large, round forms that were almost fully grown. Knowledge of the rate of growth of these parasites should indicate when they had been liberated from schizonts and, therefore, when an asexual cycle had been completed. The size of parasites in the peripheral circulation was determined therefore on successive days, beginning with the first day of patency, to obtain a measure of the rate of growth (Tables IV and V). These parasites were assumed to be potential gametocytes. Fifty or more parasites on dried and stained blood films were measured at each time interval unless otherwise stated. The average size determined for the parasites at any one time will depend on the number measured. Superimposed infections resulting from the continuous exposure of ducks would tend to reduce the average. The average would be lowered also by variation in the rate of growth, including differences in the maturation of schizonts, that is known to occur (7). An attempt was made therefore to find only the maximum rate of growth of gametocytes by selecting for comparison the 10 largest parasites that were measured at each interval. Even this may not be the exact rate of growth as the largest parasites were not necessarily observed each day. Moreover, the average size at any one time depends on the relative number of male and female gametocytes included in each sample. The latter grow to a larger size (15 μ) than the former (11 μ) and yet they cannot be distinguished readily until they are

TABLE IV

AVERAGE DIAMETER OF 10 LARGEST PARASITES (50–100 MEASURED) OBSERVED IN PERIPHERAL BLOOD OF DUCKS ON SUCCESSIVE DAYS OF PATENCY

Days after exposure	Time of measurements	Diameter (μ) of parasites and duck No.					
		1504	1505	1506	1507	1525	1561
5	11 p.m.	—	—	—	—	—	1
6	9 a.m.	4	2	3	1	—	—
	1 p.m.	3	2	3	2	2	—
	2 p.m	—	—	—	—	—	2
	6 p.m.	6	3	7	2	2	2
	10 p.m.	8	2	6	3	—	—
7	5 a.m.	10	5	7	8	4	—
	8 a.m.	—	—	—	—	4	3
	10 a.m.	12	6	9	—	—	4
	1 p.m.	—	—	—	—	4	—
	3 p.m.	10	—	—	8	—	—
	4 p.m.	—	—	—	—	—	5
	6 p.m.	—	—	—	—	7	—
	8 p.m.	14	—	11	7	—	—
	11 p.m.	12	8	11	9	—	—
8	8 a.m.	14	8	12	12	—	13
	9 a.m.	—	—	—	—	11	—
	1 p.m.	—	—	—	12	—	—
	6 p.m.	—	—	13	15	14	—
	9 p.m.	—	—	—	—	—	12
9	9 a.m.	—	—	—	—	—	13

TABLE V

PERCENTAGE OF PARASITES OF DIFFERENT SIZES ON SUCCESSIVE DAYS FOLLOWING NATURAL INFECTIONS

(Based on measurements of 30–100 parasites each day)

Day of patency	Prepatent period of 6 days						Prepatent period of 7 days		
	Duck 893			Duck 894			Duck 615		
	$< 5\mu$	$5–9\mu$	$> 9\mu$	$< 5\mu$	$5–9\mu$	$> 9\mu$	$< 5\mu$	$5–9\mu$	$> 9\mu$
1	100	0	0	100	0	0	100	0	0
2	94	6	0	99	1	0	82	18	0
3	77	9	14	84	16	0	48	24	28
4	6	2	92	12	1	87	24	14	62
5	30	3	67	23	4	73	10	0	90
6	5	30	65	—	—	—	14	7	79
7	—	—	—	—	—	—	23	7	70

about 7 μ in diameter. Parasites less than 1 μ in diameter are seen infrequently in the peripheral circulation. This is to be expected as merozoites are about this size in megaloschizonts.

The size of parasites was noted at intervals of five to nine hours in a group of six ducks that was exposed continuously to infection (Table IV). Some gametocytes attained their full size in approximately 48 hr.

A second group of ducks was exposed to infection for 36 hr. The parasites that appeared in these were measured on successive days (Table V). Measurements of the parasites, in this as in the previous group of ducks, show that some gametocytes attain their full size in 48 hr. It follows therefore that gametocytes that are fully grown five to six days after the exposure of the host must have arisen from an asexual generation that was completed at least two days previously.

Asexual Stages in Blood

Although we have not seen, or have not recognized, asexual stages of *Leucocytozoon* in the blood of infected birds, they are undoubtedly present at times as artificial transmission of the parasite has been accomplished by transfer of heart blood from an infected to a non-infected bird. Additional experiments of this type have been carried out to determine at what periods, following infection, an asexual stage of the parasite is present in blood.

Ducks were infected by exposure in an endemic area for known periods or by the injection of a suspension of infected black flies. Blood (2.5–9 cc.) was removed from the hearts of these ducks at various intervals following their exposure or following the injection of infected flies. This blood, which was prevented from clotting by heparin, was transferred as quickly as possible to parasite-free ducks. Blood smears were made from these recipient ducks 7–14 days later. The transfers that produced infections in the recipient ducks

TABLE VI

TRANSMISSION OF INFECTION WITH BLOOD FROM INFECTED DUCKS

Time from infection, hr.	No. cc. transferred	Prepatent period, days
½ – 48	5	8
1 – 28	2.5	11
27 – 51	9	?
45 – 72	5	9
41 – 65	9	?
61 – 65	5	10
65 – 95	3	?
68 – 96	2.5	10
59 – 95	6	10
69 – 105	6	10
67 – 95	3	?
51 – 75	9	< 11
66 – 78	5	8
65 – 89	?	< 10
70 – 74	5	8
83 – 119	5	9
93 – 129	3	9
106 – 109	5	16
115 – 143	3	12
117 – 153	9	8

are indicated in Table VI. Negative results are omitted, except in the one experiment that follows, since, in most instances, it was not known whether the donor ducks were infected. Blood (3 cc.) was removed, in this single experiment, from the hearts of two ducks without killing them,* at 20–48, 67–95, and 115–143 hr. after they were exposed to infection. Each of these samples of blood was injected intraperitoneally into other ducks. Infections developed in the ducks that received blood taken 67–95 and 115–143 hr. after infection but not in those that received blood taken 20–48 hr. after infection.

The results of these transfers of blood from infected to healthy ducks indicate that asexual stages are in the blood at certain times before as well as after gametocytes are detected. Dr. Garnham, in a personal communication after kindly reading our manuscript, suggested that the positive results following blood inoculations may result from merozoites, in the inoculum, growing into gametocytes as observed in experiments with *Hepatocystes* (8). This probably occurred in some of our ducks but it seems likely that asexual reproduction was initiated too since gametocytes were not observed in several ducks until 10 days after they were inoculated and never before eight days, although blood from them was examined on the seventh day. The low parasitaemias that occurred following these artificial infections, the failure to observe asexual stages in the peripheral blood, and the failure, at times, to produce infection by the transfer of blood or tissues from an infected to a healthy duck may mean that relatively few asexual parasites are free in the

*Later examination showed that they were infected.

blood. The success in initiating infections using blood that was taken from ducks 48–153 hr. after they were infected is not surprising if the length of an asexual cycle is about 48–72 hr.

An asexual cycle of this length would explain, too, some of our negative results although other explanations are possible also, for example, (*a*) all donor ducks were not necessarily infected, (*b*) asexual parasites may have been absent, or scarce, in the blood and therefore in the inoculum, (*c*) host resistance may have destroyed any that were introduced into the recipient ducks, (*d*) infections may have developed in some of the recipient ducks but they were too low grade to be detected by examining blood smears as low parasitaemias were observed in all ducks infected by blood transfers. This is in contrast to the high parasitaemias that have been observed in natural infections. We have never succeeded, in the few trials attempted, in transferring infection by blood or tissues from one of these artificially infected ducks to a third duck.

Splenomegaly and Anaemia Associated with Infection

Splenic enlargement and anaemia that occur in heavy infections have been noted (16, 12, 7). Further observations were made on splenomegaly and anaemia in relation to the time the ducks were infected to see if these pathological phenomena could be related to the asexual cycle. The size of the spleen was measured in a series of ducks that were killed at intervals of 1–16 days following their exposure to infection for 24–48 hr. (Table VII). It is apparent that the spleen shows little enlargement prior to six days after exposure, although some of these ducks may not have been infected as parasites were not observed in their blood. This is improbable as infection developed in the ducks that were kept as controls. The maximum size of the spleen was observed 10–14 days after the first exposure of the ducks to infection. This corresponds to the time when the peak parasitaemia usually occurs. If the megaloschizonts are abundant in the spleen they alone, because of their size, will account for some of the increase in the volume of the spleen. Qualitative observations on sections of spleen suggest that megaloschizonts are most numerous in the spleen 7–12 days after infection although their relative abundance in the spleen and other organs varies, of course, from one bird to

TABLE VII

SIZE OF SPLEEN (IN DUCKS 1–4 WEEKS OF AGE) IN RELATION TO INFECTION

No. measured	No. days after exposure	Spleen vol. in cc.		
		Max.	Min.	Av.
28	1– 6	1	0.1	0.3
13	7– 8	4.1	0.4	1.8
10	9–10	5.0	0.2	2.1
15	11–12	9.0	1.0	4.2
10	13–16	9.0	1.0	3.6
Eight normal ducks 1 month of age		0.6	0.3	0.4

another. Phagocytosis of merozoites is prominent following the rupture of megaloschizonts as Cowan (3) observed. This cellular response and the congestion in the spleen would be important also in producing the enlargement. These observations alone give little indication of the rate of schizogony.

Further observations were made also on the blood cell volume, haemoglobin, and number of red and white blood cells in ducks on successive days following exposure to infection for 48 hr. (Table VIII). Previous work (7) showed that a decreased cell volume, haemoglobin, and red cell count were associated with infection as well as an increase in the number of white blood cells. Consequently only the minimum or maximum values observed on successive days are given in Table VIII. A decrease in the number of red blood cells and haemoglobin, and an increase in the number of white blood cells were noted four to six days after infection. These changes were more obvious during the next 48 hr. and the abnormality continued for at least 9–11 days after infection.

Asexual Cycle as Observed in Sections of Tissues

Sections, prepared from various tissues removed from ducks at various times following exposure, were examined to determine the stages of the parasite and their sequence in the asexual cycle. Megaloschizonts have been found in brain, lung, liver, heart, kidney, gizzard, intestine, and lymphoid tissues, and no doubt they occur elsewhere as well. Hepatic schizonts were seen in liver cells removed from ducks six days after their exposure, but were not as common as megaloschizonts. The latter were noted in the tissues of some ducks four to six days after exposure. Obviously with such a widespread distribution, schizonts may be readily overlooked unless the birds have heavy infections.

Sections of liver from one duck killed three days following a 48 hr. exposure to infection revealed structures that are thought to be schizonts in Kupffer cells (Fig. 6). These were found by Dr. R. C. Ritchie, Department of Pathology, University of Toronto, while he was studying the pathology caused by this parasite. Dr. Ritchie has kindly permitted us to include an

TABLE VIII

BLOOD VALUES IN DUCKS FOLLOWING EXPOSURE TO INFECTION FOR 24–48 HR.

Days after exposure	No. examined	Min. cell vol., %	Min. Hb, gm./100 cc.	Max. W.B.C., thousands	Min. R.B.C. per cu. mm.	Schizonts
4	5	36	9.5	10.9	2.8	
5	5	36	9.5	11.6	3.0	K
6	6	32	8.5	14.2	2.9	M
7	7	22	6.0	49.3	1.6	M
8	5	23	6.0	48.1	1.9	H, M
9	7	18	4.0	32.8	1.2	H, M
10	2	25	4.3	33.1	1.5	M
11	1	17	4.0	23.0	1.1	M

K, parasite in Kupffer cell; M, megaloschizont; H, hepatic schizont.

illustration of them in this paper. If such structures are schizonts it is possible that an asexual cycle occurs in Kupffer cells prior to the appearance of hepatic and megaloschizonts. Such a cycle could provide a few gametocytes, which would account for the large forms that have been seen in a few instances in the peripheral blood of birds five to six days after they were exposed.

Discussion

The above data show that *S. croxtoni*, *S. euryadminiculum*, and *S. rugglesi* are suitable hosts for *L. simondi* and that these species of flies feed naturally on ducks. These data, together with the fact that no specimens of *S. venustum* were recovered from ducks, validate the prediction of Shewell (14) that *S. rugglesi* rather than *S. venustum* is likely the natural vector of *L. simondi*.

Sporogony in *S. rugglesi* appears to take place rapidly although no doubt the rate is variable. Such variation plus longevity of sporozoites would account for the fact that flies contained viable sporozoites for at least 18 days after the ingestion of gametocytes. Although the oöcysts are small and contain few sporozoites compared to several species of *Plasmodium* the end result may be much the same, as gametocytes of *Leucocytozoon* are often more abundant in the blood than are those of *Plasmodium*. The presence of oöcysts on the outer stomach wall and sporozoites in the salivary glands resembles the development of *Plasmodium* as O'Roke (12) pointed out. This also discredits our previous opinion (7) that sporozoites may not go to the salivary gland. However, the presence of structures regarded as oöcysts in the stomach wall, and even within the stomach, suggests that development of oöcysts is not restricted to the outer wall. Weathersby's (15) demonstration of the ectopic development of oöcysts indicates that the stomach wall is not a prerequisite for their development in *Plasmodium gallinaceum*.

It was suggested (7) that a single asexual generation may precede the appearance of gametocytes in the peripheral circulation. This may still be true but it appears from the present data that there are at least two generations of schizonts, as Cowan (3) suggests, before many gametocytes are detected. In some instances a few large gametocytes were seen in the peripheral circulation of ducks five to six days after they were exposed to infection. If these parasites developed at the maximum rate observed by us it means that a few gametocytes were produced three to four days after the ducks were exposed. There is no certainty that the ducks were infected immediately they were exposed, nor can we be sure that the parasites observed at five to six days had grown at the maximum rate. It seems likely therefore that an asexual cycle can be completed in three to four days, or perhaps even in less time. The success in producing infections by the transfer of blood from infected ducks indicates the presence of asexual parasites in the blood. These parasites could be free or within circulating cells. If schizogony requires 48–72 hr. and is somewhat variable, and if some of the merozoites are in the blood stream,

then infections should be produced with blood that was removed from ducks 48 hr. or more after they were infected. The success, in two instances, prior to this time would be expected if sporozoites were still present in the samples of blood that were transferred. This is most probable as in both instances the blood was removed from the ducks one hour or less after their last exposure to infection.

The finding, by Dr. Ritchie, of structures that are thought to be schizonts in Kupffer cells, the changes in the blood, the enlargement of the spleen, and the detection of hepatic schizonts and megaloschizonts about six days after infection, leads to the tentative conclusion that the first asexual generation occurs in Kupffer cells. Some of the merozoites from these schizonts may develop into gametocytes and thus explain the presence of a few large parasites in the blood five to six days after a bird is infected. Other merozoites from this first generation grow, presumably, into hepatic schizonts and megaloschizonts and perhaps into other schizonts in Kupffer cells. Merozoites arising from megaloschizonts, and to a lesser extent from hepatic schizonts, will account for the sudden flooding of gametocytes into the peripheral circulation beginning about six or seven days after infection. Some of these merozoites presumably develop into another asexual generation. As megaloschizonts occur in many different tissues the possibility that they arise from sporozoites should not be dismissed completely for unless they are very numerous they would not be found easily in sectioned tissues. The same argument applies to the hepatic schizonts that appear to be even less numerous and consequently may be overlooked even more readily. It may well be, as Huff (9) proposed, that the type of cell that is entered by a sporozoite or merozoite determines the kind of schizont that develops, although Cowan (4) is of the opinion that megaloschizonts are the final prepatent generation of schizonts.

As an alternative hypothesis the progeny of the first asexual generation may give rise only to another generation of schizonts. Most of the merozoites from the second and subsequent generations would grow into gametocytes and a few into another asexual generation. This seems unlikely however, as this would not explain the presence of gametocytes in the blood three to four days after infection. It is hoped that future work will resolve these questions now that *S. croxtoni*, *S. euryadminiculum*, and *S. rugglesi* have been shown to be natural hosts and apparently the natural vectors of *L. simondi*.

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THE EFFECT OF METHYL BROMIDE ON THE RESPIRATION OF THE CADELLE TENEBROIDES MAURITANICUS (L.) (COLEOPTERA: OSTOMIDAE)[1]

By E. J. Bond[2]

Abstract

Determinations have been made of the oxygen consumption of individual *Tenebroides mauritanicus* (L.) under fumigation with controlled dosages of methyl bromide. When the LD_{50} was applied, it was found that individuals characterized by a high normal respiratory rate were more likely to be victims than those with a low rate. The respiratory rate remained unchanged during the five-hour exposure to methyl bromide while the insects showed the complete succession of symptoms from hyperactivity to paralysis. Subsequently there was a gradual decrease in respiration until death. Pretreatment of the insects with a sublethal dose of the fumigant did not increase their resistance to lethal doses, thus confirming the absence of "protective stupefaction".

Introduction

The susceptibility of an insect to a fumigant is believed to be correlated with its rate of respiration. Factors which are known to increase the respiratory rate, such as high temperature, low oxygen tension, and abnormal concentrations of carbon dioxide, also increase the susceptibility of insects to fumigants (5). It remains to be established what effect the fumigant itself has on respiration.

Such studies may also shed light on the well-known phenomenon of "protective stupefaction". Insects exposed to sublethal concentrations of fumigant have been found to become refractory to lethal concentrations subsequently applied. This has been observed in citrus scale insects fumigated with hydrogen cyanide (10) and methyl bromide (25), and the granary weevil has shown a similar response to both of these fumigants (6, 16). A comparison of resistant with normally susceptible California red scale has shown that protective stupefaction in this species is connected with an increase in the duration of spiracular closure (11) and hence, presumably, a decrease in the rate of respiratory exchange. It is therefore important to determine the effect of sublethal concentrations of fumigants on the respiratory rate.

In the present investigation a method has been developed for studying the respiratory rate of an insect during fumigation; and this method has been used to determine the effect of a common fumigant, methyl bromide, on the respiration of the cadelle *Tenebroides mauritanicus* (L.). The effect of this fumigant on the respiratory rates of both larvae and adults was determined during and after fumigation. The relation of respiratory rate to the susceptibility of individual larvae and the effect of sublethal concentrations on the respiratory exchange were also determined.

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Contribution from the Science Service Laboratory (Contribution No. 78) and the Department of Zoology, University of Western Ontario, London; from a thesis approved by the University of Western Ontario for the M.Sc. degree.
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Materials and Methods

The oxygen consumption of the insects was measured in the Warburg constant volume respirometer using double sidearm flasks of 20 ml. capacity. One insect was placed in the moat of each flask. Following the method of Umbreit *et al.* (23), the fumigant–air mixture was pumped through the stopcock of the manometer into the flask and out through the venting tube in the sidearm so that the atmosphere within the flask was completely replaced by the mixture. A manifold was used to supply gas to any or all of the flasks simultaneously. After the mixture was flushed through the system for 15 min. and another 15 min. was allowed for temperature equilibration, the system was closed and the oxygen consumption of the insects was measured for the balance of the five-hour fumigation period. The carbon dioxide produced by the insects was absorbed on a strip of filter paper moistened with 0.2 ml. of 20% potassium hydroxide. All experiments were conducted at 25° C. and 70 % R.H.

The fumigant–air mixture was prepared by the method described by Monro and Buckland (18) in which methyl bromide was introduced from a steel cylinder into a 525-liter chamber. The dosage was attained by measuring on a manometer a calculated pressure increase corresponding to the particular weight of fumigant required; this dosage was checked by weighing the cylinder and by analyzing the contents of the chamber. The fumigant–air mixture was then pumped by an airtight diaphragm-type pump* through polyethylene tubing (3/16 in. I.D.) to the manifold and the flasks. The concentration of methyl bromide flowing through the flasks was analyzed at the venting tube by a thermal conductivity gas analyzer (19). It was found that the 15-min. period of flushing the mixture so covered the inner surfaces of the flasks that adsorption of fumigant taking place subsequent to closing of the system decreased the gas volume by only seven microliters. Two thermobarometers were used in each experiment; one contained the mixture, the other air only.

The test insects were reared according to the method previously described (3). Full-grown fourth-instar larvae were used in most experiments since they were large and had reached a more uniform state of development. When adults were used they were taken one week after emergence and before mating had taken place. The insects were placed individually in the flasks the evening previous to fumigation.

The possibility that the insects themselves removed significant quantities of methyl bromide by sorption to their bodies was checked by fumigating 50 larvae in a 1000 ml. flask at the LD_{99} level for five hours. Analysis of the atmosphere within the flask by the thermal conductivity analyzer showed the concentration, after a five-hour exposure period, to be similar to that in a control flask containing no insects.

Larvae were exposed for five hours to three concentrations of methyl bromide: the LD_{99} (23 mgm./l.); the LD_{50} (16 mgm./l.); and the maximum sublethal dose (10 mgm./l.). Adults were exposed to the LD_{99}, which was

**Made by Charles Austin Ltd., West Malling, Kent, England.*

TABLE I

BODY WEIGHT AND OXYGEN CONSUMPTION (μL./INSECT) OF THE 10 LARVAE WITH THE HIGHEST RESPIRATORY RATES AND OF THE 10 WITH THE LOWEST RESPIRATORY RATES, SELECTED FROM A SAMPLE OF 42 LARVAE TO SHOW THAT THERE IS NO MARKED RELATIONSHIP BETWEEN BODY WEIGHT AND OXYGEN CONSUMPTION

Low O_2 consumption		High O_2 consumption	
Wt. (mgm.)	O_2 consumption (3 hr.)	Wt. (mgm.)	O_2 consumption (3 hr.)
46.5	68.9	43.3	140.7
55.8	70.8	49.8	150.4
39.7	70.6	44.5	153.8
46.0	76.6	55.1	172.8
53.1	77.4	42.0	186.5
41.4	78.6	48.4	188.8
52.4	81.4	46.8	195.3
42.0	84.5	49.5	230.9
56.4	86.2	54.0	237.0
47.7	86.6	50.8	310.4
48.1 ± 1.9	78.2 ± 2.1	48.4 ± 1.4	196.7 ± 11.6

23 mgm./l., as for the larvae (17). The oxygen consumption was determined every 30 min., not only during the fumigation period, but also for three hours before the fumigant was added. In the case of adults and larvae exposed to the LD_{99} dosage, readings were continued for 12 hr. after removal of the fumigant.

All results on oxygen consumption are expressed in terms of the whole insect because the oxygen consumption of the larva, the main test stage, does not appear to be related to its body weight, but rather seems to be peculiar to each individual (Table I). The oxygen consumption among individual larvae varied as much as four times from the lowest to the highest. The difference in respiratory rates between treated and untreated insects was tested for significance by Student's t test. The distribution of t was obtained from the statistical tables of Fisher and Yates (8).

Results

Relationship Between Susceptibility and Rate of Respiration

When larvae of the cadelle were treated at the LD_{50} level the survivors were found to have a significantly lower rate of respiration than those that succumbed, and this difference was present before as well as during fumigation. The respiratory rates of a group of 42 insects were assessed for a three-hour period just after removal from the food (20 hr. before fumigation), just before fumigation, and for four and one-half hours during the exposure period; it was found that the average respiratory rate of the survivors was

lower than that of the victims at each assessment. The average values for oxygen consumption of each of the two groups, in microliters per insect per three hours were as follows:

	Time of assessment		
	−20 hr.	0 hr.	+5 hr.
Victims	208.2 ± 18.3	143.1 ± 9.5	136.1 ± 8.5
Survivors	157.5 ± 10.3	98.7 ± 5.3	103.5 ± 5.0

The values for the individual insects are plotted and joined by lines to show the change in rate at each assessment (Fig. 1), those values for the four and one-half hour fumigation period have been adjusted to conform with the three hour period otherwise employed. It should be noted that while the respiration of most larvae declined after removal from the food

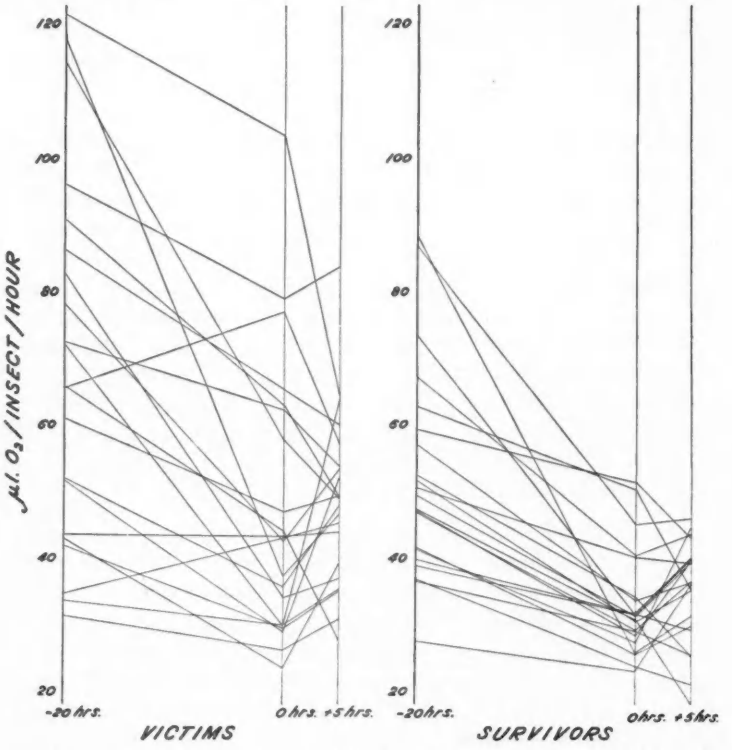

FIG. 1. Diagram showing the variability in respiratory rates of individual larvae at the time they were removed from the food (−20 hr.), just before (0 hr.), and during (+5 hr.) exposure to methyl bromide at the LD_{50} level (16 mgm./l.).

(−20 hr. to 0 hr.) the application of the fumigant caused the respiration of some to increase and of others to decrease in varying degrees, thus showing further the individuality of the larvae.

The possibility that this difference in respiratory rates was due to a difference in sex was checked by determining the oxygen consumption of 64 fourth-instar larvae and then determining their sex when they had reached the pupal stage. The average oxygen consumption in a three hour period for 25 male larvae was 121.6 ± 6.9 and for 39 female larvae 129.3 ± 7.2. The difference between the two rates is not significant ($t = 0.726$), thereby showing that the difference in respiration between the victims and survivors of a fumigation cannot be attributable to a difference in sex.

Effect of Lethal Concentrations on Larval Respiration

Fumigation at the LD_{50} and LD_{99} levels did not significantly change the respiratory rates of fourth-instar larvae while they were under methyl bromide vapor. Statistical analysis of the data showed no significant difference

TABLE II

AVERAGE OXYGEN CONSUMPTION (μL./INSECT) DURING 4½ HR. BY LARVAE EXPOSED TO A 5-HR. FUMIGATION WITH VARIOUS DOSAGES OF METHYL BROMIDE

	LD_{99}	LD_{50}	Max. sublethal dose	Control
	163.1 ± 8.0	181.8 ± 11.8	163.8 ± 8.7	168.6 ± 8.9
t_{52} (Student's test)	0.463	0.951	0.358	—

TABLE III

BEHAVIOR OF THE CADELLE (7 INSECTS PER TEST) AT THE END OF A 5-HR. EXPOSURE TO AN LD_{99} (23 MGM./L.) OF METHYL BROMIDE

	Behavior	Average O_2 consumption (μl./min./insect)	
		Just before exposure	During final 30 min. of exposure
Male adults	7 insects on backs, occasional movement of appendages	0.23	0.20
Female adults	6 insects on backs, occasional movement of appendages; 1 insect upright, no movement	0.22	0.25
Larvae	5 insects on backs, rolling from side to side; 1 insect on side and 1 upright, neither moving	0.53	0.47

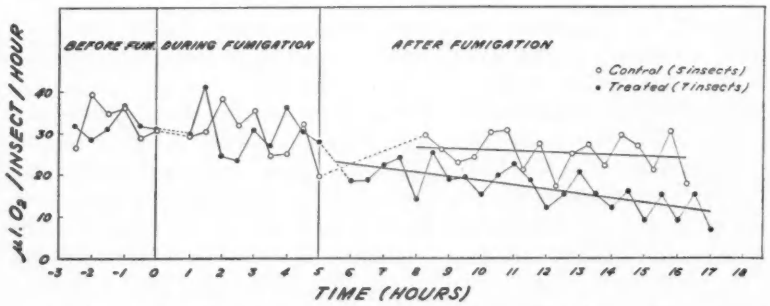

FIG. 2. The pattern of oxygen consumption of larvae before, during, and after exposure to methyl bromide at the LD_{99} level (23 mgm./l.), with regression lines calculated from the values obtained after fumigation.

between the rate of oxygen consumption of untreated larvae and that of larvae treated either at the LD_{99} or the LD_{50} level (Table II). However the activity of the fumigated larvae was considerably affected, as observations at the LD_{99} level showed; they were initially stimulated to abnormal activity and then became progressively more ataxic until complete paralysis took place four to five hours later (Table III). The respiratory rates of these larvae were found to decline steadily, so that 12 hr. after fumigation they were about one half that of the normal (Fig. 2).

Response of Larvae to a Sublethal Concentration

When the larvae were treated with the maximum sublethal dose of methyl bromide there was no significant change in their respiratory rates during the exposure period (Table II). Therefore the larvae do not enter a state of respiratory depression whereby the intake of the fumigant–air mixture is reduced. The possibility that any protective effect was induced by exposure to the fumigant was further studied by pretreating the larvae for one, two, and three hours with the maximum sublethal dose before subjecting them to a lethal dose. The results from this experiment showed that the mortality among insects pretreated for one hour was significantly greater than among those receiving no pretreatment, and the mortality was even higher among those pretreated for two and three hours (Table IV). Therefore the sublethal

TABLE IV

THE MORTALITY RESULTS OF CADELLE LARVAE PRETREATED WITH A MAXIMUM SUBLETHAL DOSE BEFORE BEING SUBJECTED TO A LETHAL TREATMENT

Pretreatment period (hr.)	Pretreatment dosage (mgm./l.)	Treatment dosage (mgm./l.)	C × T Factor	% Mortality
0	—	23.0	115.0	81.3
1	9.9	22.0	119.9	88.0
2	9.7	22.4	131.4	91.9
3	9.7	21.5	136.6	98.3

TABLE V

AVERAGE OXYGEN CONSUMPTION (μL./INSECT) OF TREATED AND UNTREATED ADULTS FOR 4 HR. DURING A 5-HR. EXPOSURE TO METHYL BROMIDE AT THE LD_{99} (23 MGM./L.) LEVEL

Females		Males	
Treated	Control	Treated	Control
65.9 ± 2.5	61.8 ± 3.0	58.8 ± 2.4	53.7 ± 2.1
t_{39} = 1.030		t_{28} = 1.590	

treatment did not enhance the resistance of this insect to the fumigant. Hence the phenomenon of "protective stupefaction" which has been observed in other insects when fumigated with methyl bromide does not occur in the cadelle.

Effect of a Lethal Concentration on Adult Respiration

When adult cadelles were treated with methyl bromide at the LD_{99} level the rates of oxygen consumption were not significantly affected (Table V); the pattern of uptake was similar for treated and untreated insects of either sex (Fig. 3). However, as already observed with larvae, the treatment considerably affected the activity of the adults (Table III). They became progressively more active during the first two hours of exposure, finally to fall on their backs; thereafter, their activity gradually decreased until only

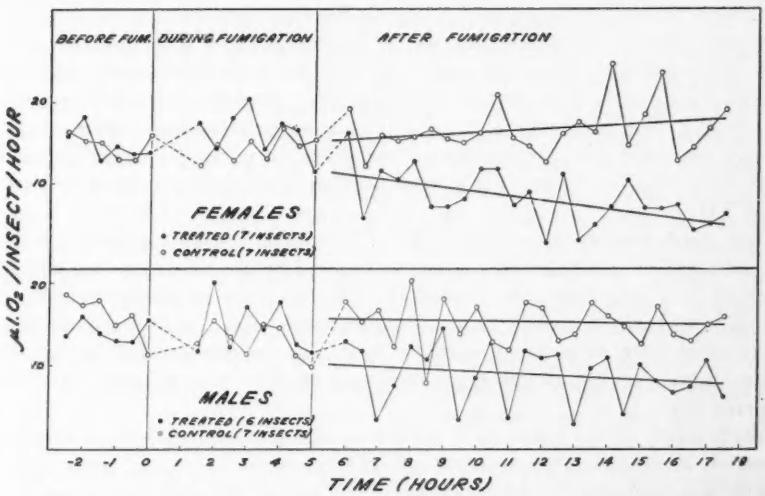

FIG. 3. The pattern of oxygen consumption of male and female adults before, during, and after exposure to methyl bromide at the LD_{99} level (23 mgm./l.), with regression lines calculated from values obtained after fumigation.

occasional movements of the appendages could be observed at the end of the exposure period. The respiratory rates for the 12-hr. observation period, after fumigation, showed a slow and steady decline (Fig. 3). Regression lines showed the rate of decline to be 2% per hour for the males and 5% per hour for the females.

TABLE VI

A COMPARISON OF OXYGEN CONSUMPTION (μL./HR.) RATES EXPRESSED IN TERMS OF THE WHOLE INSECT AND OF BODY WEIGHT

	O_2 uptake	
	Per insect	Per gm. body wt.
Male adults	13.4 ± 0.5	542.2 ± 19.3
Female adults	15.5 ± 0.8	506.9 ± 25.3
Larvae	37.5 ± 2.0	786.3 ± 39.3

Discussion

The observation that the larvae which respired most were those which showed the highest mortality suggests that higher gaseous exchange enhances the toxicity of methyl bromide vapor. The greater exposure of tracheae to the fumigant and the higher metabolic rate could confer susceptibility. This assumption is supported by the finding of Jones (12) that carbon dioxide, which causes the spiracles to remain open, increases the toxicity of methyl bromide to *Tribolium castaneum* (Hbst.).

Although the stage of an insect which shows little gaseous exchange, such as the pupa, is less susceptible than an actively respiring stage, Lindgren (15) found that adults of *Tribolium confusum* Duv. were slightly less susceptible to carbon disulphide than larvae although they respired more per unit of body weight. On the other hand, the present experiments have shown that although the adults of *Tenebroides mauritanicus* were just as susceptible as larvae, their respiratory rate per unit of body weight was lower by one-third (Table VI).

When three species were compared in the adult stage, Lindgren found that, although *T. confusum* had a higher rate of respiration than *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.) it was more resistant to carbon disulphide and ethylene oxide than the latter two species. However, toxicity is correlated with respiratory rate in *Sitophilus* in that *S. oryzae*, which respires more rapidly, is generally more susceptible to fumigants than *S. granarius* (15).

Susceptibility of an insect to a particular fumigant depends, to a certain extent, on its respiratory rate when initially exposed to the gas. Protective stupefaction, probably due to a decrease in tracheal ventilation, is the response shown by certain species to the exposure to a sublethal dose, before the lethal dose penetrates to the insects. In normal fumigations at atmospheric pressure

the maximum concentration of methyl bromide in the center of a sack of wheat is not reached until one hour after application of the fumigant (7). It is this condition that allows the insect to reach the stupefied state and thus evade the lethal effects of the fumigant. Since the cadelle showed no significant decrease in respiration when exposed to a sublethal dose, and since its susceptibility was not markedly decreased by this exposure, it is evident that protective stupefaction is not involved when this species is fumigated with methyl bromide. The inability of lethal doses to decrease the respiratory rate during the fumigation period also indicates that tracheal ventilation has remained unaffected.

Methyl bromide is considered to be an irritant poison for insects. Brown and Reynolds (private communication reported by Winteringham and Barnes (24)), could not detect any anesthesia in adults of *T. confusum* and *S. granarius*; instead, severe poisoning produced spasmodic and uncoordinated movements which became feebler and more infrequent as death approached. Shepard and Buzicky (21) found that adults of *T. confusum* appeared quite healthy after a five hour exposure to methyl bromide, although they were all to die within 48 hr. In recent experiments with methyl bromide (at the LD_{99} level) the author observed *T. confusum* to respond merely by a depression in activity, whereas *Musca domestica* L. showed slight signs of irritation before suppression of activity became evident. The present study shows *T. mauritanicus* in sharp contrast to the above species for the adults are paralyzed during the fumigation period and the larvae soon after. The paralysis was preceded by a period of hyperactivity without convulsions, and thus the symptoms showed the progression—excitation, paralysis, death—, which is cited as typical for narcotic fumigants (4).

Methyl bromide has several properties characteristic of narcotic fumigants. It is highly soluble in lipoids. Like many well-known narcotics, it is a volatile halogenated hydrocarbon. It has been found to inhibit succinic dehydrogenase (14), and thereby resembles narcotics such as urethane, chloroform, alcohol, and ether, which are well-known inhibitors of dehydrogenase systems (1, 2). However, methyl bromide is unlike the narcotics in that its effects are slow in taking place. Whereas narcotic fumigants such as carbon tetrachloride and carbon disulphide induce paralysis in the aphid *Macrosiphum tulipae* Fonsc. in one minute (13), methyl bromide does not paralyze the cadelle until the fourth hour of fumigation. Furthermore, methyl bromide does not affect the respiratory rate of this insect during fumigation; narcotics, on the other hand, are characterized by their inhibition of respiration even at low concentrations (2). This depression is usually reversible with narcotics, but with methyl bromide the cadelle is irreversibly paralyzed before its respiration is significantly decreased.

It is remarkable that the respiratory rates of the insects remain constant during the successive symptoms of poisoning from normal to hyperactivity to paralysis. The absence of a significant increase in oxygen consumption during the hyperactive stage may be attributable to a partial inhibition of

some phase of oxidative metabolism. Methyl bromide is known to inhibit succinic dehydrogenase (14), an essential part of the tricarboxylic acid cycle, and could thus interrupt the cycle and reduce oxidative metabolism. A reduction of oxidative metabolism has been found to stimulate glycolysis in the insect *Carpocapsa* (9), and it is thus possible that the glycolytic processes could supply the extra energy required for hyperactivity. The irreversibility of poisoning once the paralytic stage is reached may be due to irreversible inhibition of the enzyme by methyl bromide.

The data presented in this paper show that the susceptibility of individual cadelles to methyl bromide is correlated with their characteristic rates of oxygen consumption. They also indicate that the cadelle does not exhibit protective stupefaction, thereby differing from the granary weevil and certain scale insects. Furthermore, *T. mauritanicus* differs from *Tribolium*, *Sitophilus*, and *Musca* in being paralyzed during fumigation by methyl bromide. These results serve to demonstrate that various insects respond to insecticides in different ways and that observations on a number of species are required to provide a full understanding of insecticidal action.

Acknowledgments

The suggestions and criticisms of my supervisor, Prof. A. W. A. Brown, Head, Department of Zoology, University of Western Ontario, have been invaluable. I am indebted to Dr. H. Martin, Director, Science Service Laboratory, for providing facilities and giving encouragement to carry out this work and to Mr. H. A. U. Monro, Senior Officer of the fumigation section of this laboratory, for helpful advice and encouragement. Valuable technical assistance was given by Mr. C. T. Buckland and Mr. E. Upitis. Advice on sorption problems was freely given by Mr. R. A. Latimer and Dr. D. M. Miller.

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AN INVESTIGATION OF TICKS AS DISEASE VECTORS IN BANFF NATIONAL PARK, ALBERTA[1]

By A. W. F. Banfield[2]

Abstract

A collection of 245 ticks (*Dermacentor andersoni*, Stiles, and *Dermacentor albipictus*, Packard) was made in the spring of 1953, from 17 localities in Banff National Park, Alberta. Laboratory tests indicated the presence of Colorado tick fever virus in three of 17 lots examined. One complement-fixation test was significantly positive for the Rocky Mountain spotted fever *Rickettsia* out of 19 tests. There was no indication of tularemia. One determination of the Q-fever virus was probably caused by laboratory contamination. The unfed adult ticks *(D. andersoni)* were found to be locally active from April 20 to June 22, 1953. The bighorn sheep *(Ovis canadensis)* was found to be the common local host of the adult tick. The Columbian ground squirrel *(Citellus columbianus)* was found to be the common host of the nymphs.

Introduction

A number of collections of ticks *(Dermacentor andersoni*, Stiles) from Banff National Park, Alberta, have been tested for the occurrence of *Dermacentroxenus rickettsia*, the causative organism of Rocky Mountain spotted fever. The Alberta Department of Public Health personnel collected ticks in 1939, 1941, 1943, 1945, 1947, and 1952 near the Vermilion Lakes, four miles west of Banff. The National Parks Service collected a few ticks at the Tumble Creek camp ground, 107 miles north of Banff in 1949.

These collections were sent to Dr. F. A. Humphreys, Laboratory of Hygiene, Department of National Health and Welfare, Kamloops, B.C. All collections prior to 1952 were negative. Nineteen ticks collected at three stations near the Third Vermilion Lake on May 8 and 9, 1952, were reported to be positive by Dr. Humphreys. These ticks had been pooled and inoculated into a guinea pig. The test animal developed lesions and a fever attributed to Rocky Mountain spotted fever, but recovered. A month later it was challenged with two inoculations of live virus (a strain usually considered 80% fatal). The animal showed no ill effects. It was therefore concluded that the animal had previously contracted the disease and developed an immunity.

Investigation Objectives

An investigation was undertaken for the Canadian Wildlife Service in the spring of 1953. The objectives were to obtain more information on the incidence, distribution, and epidemiology of Rocky Mountain spotted fever in the park, and to determine whether the ticks harbored other pathogens as well.

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Contribution from the Canadian Wildlife Service, Department of Northern Affairs and National Resources, Ottawa, Canada.
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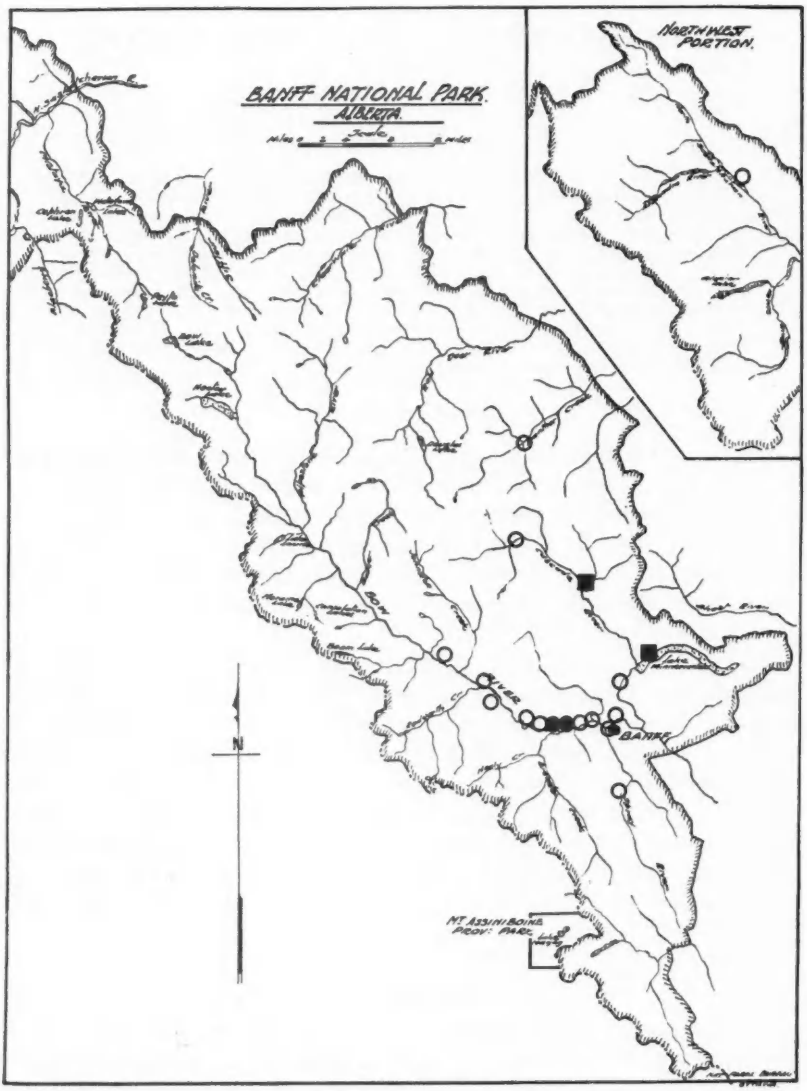

FIG. 1. Location of areas sampled and sites of positive determinations of Colorado tick fever and Rocky Mountain spotted fever.

○ Location of areas dragged for ticks.
● Localities where Colorado tick fever was recorded.
■ Locations of positive complement-fixation test for spotted fever.

Field Methods

Nineteen days were spent in the field between April 20 and June 22, 1953, collecting ticks. Seventeen areas in Banff National Park (indicated in Fig. 1) and four areas in Jasper National Park were examined. No ticks were secured from the Jasper Park localities—the Pallisades road, Pyramid Lake, Henry House flats, and the Fish Hatchery road, which were sampled in early June.

Thirty-six lots of live unfed ticks were collected in Banff Park. These totalled 245 ticks including 238 *D. andersoni* and seven *D. albipictus*, Packard (Table I).

Various types of game habitat from the valley floor (elevation 4500 ft.) to the mountain slopes (elevation 6000 ft.) were sampled. The habitats included rocky slopes, grassy slopes, aspen *(Populus tremuloides)*, and Douglas

TABLE I

LOCATION AND NUMBER OF TICKS COLLECTED

Lot No.	Date	Location	Habitat	Tick numbers
1	April 22	Highway No. 1 west mile 3	Rocky hillside	5
2	" 22	" " 1 " " "	" "	9
3	" 24	" " 1 " " 7	Grassy hillside	8
4	" 24	" " 1 " " 6	Rocky hillside	6
5	" 24	" " 1 " " 3	(Nymphs)	6
6	May 5	" " 1 " " 4	Aspen grove slope	3
7	" 5	" " 1 " " 5	Rocky hillside	9
8	" 5	" " 1 " " "	" "	12
9	" 5	" " 1 " " "	" "	19
10	" 5	" " 1 " " "	" "	23
11	" 5	" " 1 " " "	" "	15
12	" 5	" " 1 " " 2	(Nymphs)	6
13	May 6	Highway No. 1 west mile 2	Douglas fir slope	1
14	" 6	" " 1 " " 3	Rocky hillside	5
15	" 6	" " 1 " " "	" "	6
16	" 6	" " 1 " " "	" "	6
17	" 6	" " 1 " " "	(Incl. 2 *D. albipictus*)	6
18	" 6	" " 1 " " "	Rocky hillside	6
19	" 6	Spray River road " 7	*(D. albipictus)*	2
20	" 6	Highway No. 1 west mile 1	Nymphs	8
21	" 6	Banff	(*D. albipictus* from deer)	1
22	" 8	Bankhead meadow	Nymphs	2
23	" 9	Tunnel Mt. Campground	(Incl. 2 *D. albipictus*)	3
24	" 19	Lake Minnewanka shore	Rocky hillside	6
25	" 19	Palliser slope	Grassy slope	9
26	" 25	Highway No. 1 west mile 7	Rocky slope	2
27	" 25	" " 1 " " 5	" "	12
28	" 25	" " 1 " " "	" "	2
29	" 25	Graveyards cabin	Wet meadow	1
30	" 28	Hillsdale meadow	Dry meadow	1
31	June 1	Flints Park	Rock talus	9
32	" 1	" "	Rock talus	7
33	" 1	" "	" "	7
34	" 1	" "	" "	14
35	" 10	Highway No. 1 west mile 8	Aspen grove	6
36	" 22	" " 1 " " 3	" "	2
37	July 30	Windy Cabin	Dry meadow	0
				245

fir *(Pseudotsuga taxifolia)* groves, willow *(Salix* sp.) thickets, wet and dry meadows. These areas included the winter ranges of such big game species as: elk *(Cervus canadensis)*, mule deer *(Odocoileus hemionus)*, moose *(Alces alces)*, bighorn sheep *(Ovis canadensis)*, and mountain goat *(Oreamnos americanus)*.

The flat unfed ticks were obtained by dragging a four-foot square of gray woolen blanket, laced to a pole, behind the investigators. Sample areas of about 10 acres were systematically covered by two men, in transects about 10 ft. apart. Every few minutes the drags were examined and the ticks found clinging to the blanket were placed in numbered vials.

The vials of live ticks were sent to Dr. William L. Jellison, Rocky Mountain Laboratory, U.S. National Institute of Health, Hamilton, Montana, where the laboratory tests were undertaken.

At the same time as the dragging operations were conducted, a number of chipmunks *(Eutamias minimus)*, and ground squirrels *(Citellus columbianus* and *Citellus lateralis)*, were collected and examined for external parasites. A further series of ground squirrels was examined in late July and August prior to hibernation.

Field Observations

The Rocky Mountain spotted fever tick *(D. andersoni)* was found to be widely distributed throughout the park in suitable habitats, during the spring months. It was not found in Jasper National Park in the present study although it has been reported there previously (Brown and Kohls (2)). Perhaps we visited the area too late to observe the maximum populations.

The ticks were found to be fairly common on rocky and grassy hillsides frequented by bighorn sheep or mountain goats. In these locations the ticks were most plentiful along the game trails, and on flat rocks. A few were found on low shrubs such as juniper (*Juniperus* sp.), willows, birch *(Betula glandulosa)*, and bearberry (*Arctostaphylos* sp.). Ticks were rare or absent in wooded regions, willow thickets, and valley meadows. These areas are frequented by large numbers of elk, moose, and deer during the winter and spring months.

The first active ticks were seen on April 20, 1953, at a time when there were still extensive patches of snow on the ground. The last tick was collected on June 22, 1953. A further sweep on July 30 failed to produce a tick.

Two chipmunks, six Columbian ground squirrels, and one golden-mantled ground squirrel were collected for examination in May from the Saskatchewan and Bow Valleys. All the Columbian ground squirrels were infested with nymphs of *D. andersoni*. None of the other species were infested. None of the seven Columbian ground squirrels and one chipmunk collected in July and August were infested with nymphs.

A number of small mammals had been collected in the park during September and October, 1952. These included: one dusky mountain shrew *(Sorex obscurus)*, one water shrew *(Sorex palustris)*, three white-footed mice

(Peromyscus maniculatus), 11 red-backed voles *(Clethrionomys gapperi)*, three phenacomys *(Phenacomys intermedius)*, and three meadow voles *(Microtus pennsylvanicus)*. Routine examination of these specimens failed to disclose any "seed" ticks, although other external parasites were collected. These animals, however, did not come from the areas where nymphs and adult ticks were later obtained.

Green (4) reported that six magpies *(Pica pica)* collected from the bighorn sheep range contained engorged spotted fever ticks in their stomachs. During the present investigation, on two occasions magpies were observed perched on the backs of elk and pecking at objects in the back pelage. Four magpies were collected on the sample areas. None of these contained ticks.

Laboratory Results

Dr. Jellison reported that various methods were used to demonstrate infection in the ticks. These included feeding on guinea pigs, inoculation into guinea pigs and white mice, complement-fixation tests of the experimental animals' blood, and finally, challenging the guinea pigs with spotted fever, *Rickettsia*. Four separate laboratories conducted some part of the tests. The laboratory results are summarized below.

Colorado Tick Fever

Dr. Carl Eklund reported the recovery of the Colorado tick fever virus from lots 6 and 17, and from a pool of ticks from lots 9 and 11. Seventeen other lots tested were negative for this pathogen. The positive lots came from three stations on the mountain slope at mile posts 3, 4, and 5 west of Banff. These stations were on slopes occupied by bighorn sheep. The location of these stations is given in Fig. 1.

Q-fever

One guinea pig, which was inoculated with a pool of ticks from lots 1, 2, 3, 4, and 5, gave a positive complement-fixation test of 4+ at 1:64 for Q-fever. Its mate, which received the same inoculum, was negative. Thirteen other tests were consistently negative. Dr. Lackman believed that this was a case of laboratory infection. These are frequent and unavoidable.

Rocky Mountain Spotted Fever

Nineteen guinea pigs acted as hosts to the ticks and were inoculated with pooled ticks. None of these developed frank spotted fever within 20 days. One developed a post inoculation two-day fever without definite lesions.

Complement-fixation tests were run on blood samples from these animals. Six tests were positive at a low titer of 4+ at 1:16. All these animals developed a typical course of spotted fever when challenged with live *Rickettsia*. This would discount the suggestive results of the complement-fixation tests.

The test, however, was significantly positive for one animal at a titer of 1:64. It had been inoculated with a pool of ticks from lots 24 and 25. Its

mate, however, which had received a portion of the same inoculum, remained negative. These lots were collected on the north shore of Lake Minnewanka and on the Mount Palliser slope (Fig. 1). Both these areas are frequented by bighorn sheep. Inconclusive tests were obtained on ticks collected at mile posts 3 and 4, west of Banff (the sites of the 1952 determinations).

Tularemia

None of the 19 experimental animals which were inoculated developed lesions attributed to tularemia.

Discussion

Dr. Jellison hesitated to accept these complement-fixation tests as positive evidence of the occurrence of the spotted fever pathogen in the park. He pointed out that similar inconclusive determinations had been made elsewhere outside the range of the disease.

The identification of the Colorado tick fever virus is the first recovery of this pathogen in Canada. This record was briefly reported by Eklund, Kohls, and Brennan (3). Brown (1) reported confirming records from the Banff and Brooks areas of Alberta in 1954.

It is unfortunate that a number of the field lots were pooled for testing. This meant that ticks from two or more locations were grouped. This made the accurate determination of pathogen distribution and incidence impossible. Future collections for similar studies should be made in lots of equal number from separate locations in order to determine incidence. These lots should be tested independently to determine distribution.

The most important host of the adult ticks in the area under study seems to be the bighorn sheep. The most important host of the nymphs appears to be the Columbian ground squirrel. These are infested at the same time that the adult ticks are active. The squirrels seem to be free of ticks during the summer prior to hibernation.

The collection of unfed adult winter ticks *(D. albipictus)*, seeking hosts in spring, was unexpected. These specimens were not tested for pathogens. The nymph ticks were not tested either.

The National Parks Service has undertaken a program of spraying the camp grounds near the Vermilion Lakes, Mount Eisenhower, and Tumble Creek with D.D.T. solution several times during the spring "tick" season to lessen the chance of human contact.

Acknowledgments

It is a pleasure to acknowledge the valuable assistance of Dr. W. L. Jellison and other staff members of the Rocky Mountain Laboratory, Hamilton, Montana, in laboratory determinations. Mr. J. H. Brown, Alberta Department of Public Health, supplied the information on their previous surveys. I wish also to acknowledge the assistance of Mr. R. Webb, presently with Alberta Department of Lands and Forests, in the field work.

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STUDIES ON THE GENUS KALICEPHALUS (NEMATODA: DIAPHANOCEPHALIDAE)

I. ON THE LIFE HISTORIES OF THE NORTH AMERICAN SPECIES K. PARVUS, K. AGKISTRODONTIS, AND K. RECTIPHILUS[1]

By G. A. Schad[2,3]

Abstract

The life histories of *Kalicephalus parvus*, *K. agkistrodontis*, and *K. rectiphilus* were investigated experimentally. Differences between the three species are noted in histotropism and rate of development. The parasitic third stage of *K. parvus* is given special attention, as is the development of its genital and spicular primordia. Infection per os was successful in all three species. Skin penetration may also occur. The mode of infection in nature is discussed.

Introduction

This paper is the first of a series of publications planned to report the author's investigations on the genus *Kalicephalus*, bursate nematodes of snakes. A comprehensive treatment of the genus is planned and subsequent publications will contribute toward its taxonomic revision.

Two nematode genera, *Kalicephalus* Molin, 1861 and *Diaphanocephalus* Diesing, 1851, form the small family Diaphanocephalidae. Members of this family are bursate and are characterized by laterally compressed, bivalved buccal capsules bearing internally one or two transverse cuticular ridges and externally three parenchymatous bands terminating in the circumoral papillae. They are parasitic in reptiles where, attached to the walls of the digestive tract, they feed on the host's tissues.

The genus *Diaphanocephalus*, confined to South American lizards, is comprised of two species, *Diaphanocephalus galeatus* (Rudolphi, 1819) and *Diaphanocephalus diesingi* de Freitas and Lent, 1938. Structural characters of the buccal capsule separate this genus from the closely related *Kalicephalus*.

The latter, composed of approximately 50 currently recognized species, is normally parasitic in snakes and occurs in all sections of the digestive tract from oesophagus to rectum. Species of *Kalicephalus* have been recovered from snakes inhabiting all zoogeographical regions and all major habitats.

Taxonomically the genus *Kalicephalus* is difficult. The established systematic characters are frequently more variable within a species than they are between two species described as distinct. The taxonomic problems facing a student of *Kalicephalus* today are not only those inherent in the genus. These difficulties have been greatly multiplied by inadequate descriptions, often based on few specimens or on males or females alone, and by the failure

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Contribution from the Institute of Parasitology, McGill University, Macdonald College P. O., Que., Canada, and from the Biological Research Institute of the San Diego Zoological Society, with financial assistance from the National Research Council of Canada and the Biological Research Institute, San Diego Zoological Society, San Diego, Calif.
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of most authors to restudy type material of early workers. The resultant chaos has led even the most reliable nematologists into error and today one is confronted by a tangle of species which are most difficult to separate.

Therefore, when attempting to identify several collections of *Kalicephalus* from snakes dying at the New York Zoological Park, the author found it necessary to undertake studies toward a complete revision of the genus. A project involving taxonomy, life history, variability, and geographical distribution has developed. This first paper will report life history studies on three North American species.

Historical Review

Previous publications on the genus *Kalicephalus* have dealt almost exclusively with systematics. A review of this work will be deferred for inclusion in a subsequent paper taxonomic in scope.

The life history of no kalicephalid is known completely. However, Ortlepp (13) published an account of the free-living stages of *K. philodryadus*. He described this species from the stomach, duodenum, and intestine of the South American snake *Philodryas serra*. His observations on the development of this species may be summarized as follows:

Eggs laid in advanced segmentation develop and hatch in tap water. The free-living larval stages, resembling those of other bursate nematodes, develop into the third stage in five days. These are ensheathed and sluggish.

All further work was negative (i.e. infection experiments, skin penetration experiments, etc.) and this, in Ortlepp's opinion, was attributable to the weakness of the larvae, which were cultured in tap water and thus deprived of nutrients.

Other than Ortlepp's account, there are only incidental observations available in the literature. Harwood (10) mentions the larvae of *K. agkistrodontis* in four soil types and remarks further that these undergo appreciable development in aqueous culture.

Materials and Methods

All parasitic material used in the life history studies reported here was originally collected from Black Racers, *Coluber constrictor constrictor*, purchased from Ross Allen's Reptile Institute, Florida. *K. parvus* and *K. rectiphilus* were maintained in gopher snakes, *Pituophis* sp.; *K. agkistrodontis* was not maintained in the laboratory but collected directly from the purchased snakes.

Kalicephalus-free snakes were obtained as follows:

1. *Pituophis catenifer annectans* were reared from eggs laid by captive gopher snakes at the San Diego Zoological Park. These eggs were hatched in earthenware crocks under helminthologically sterile conditions.

2. Wild *Pituophis* spp. were obtained from the Arizona-Sonora Museum. By survey it was found that American desert snakes were free of *Kalicephalus* spp. Similarly, *Thamnophis sirtalis* and *Storeria dekayi* from Ste. Anne de

Bellevue, Quebec, were known from previous experience to be free of *Kalicephalus* spp. However, faecal examinations were conducted on both groups before any snake was considered negative.

3. *Thamnophis sirtalis* and *Storeria dekayi* born in the laboratory were maintained under helminthologically sterile conditions from birth.

Tap Water Cultures

Eggs obtained from female kalicephalids by dissection were introduced into shallow tap water in a Petri dish. Many larvae from these cultures died before reaching the third stage, and those which did reach the infective stage gave variable results in infection experiments.

Sand–Charcoal–Faeces Cultures

Faeces collected from an experimentally infected snake (a pure infection), helminthologically sterile sand, and charcoal were mixed in equal quantities and made into a moist culture with tap water. This method proved most satisfactory; invariably numerous vigorous larvae were produced. Larvae were recovered from sand–charcoal–faeces cultures by the Baermann apparatus.

Infection Techniques

(*a*) *Oral*

Larvae were introduced orally by either a blunted Pasteur pipette or by a hypodermic syringe, the needle of which was sheathed by catheter tubing.

(*b*) *Percutaneous*

Snakes to be used for these experiments were restricted from water until immediately before experimentation, when they were allowed to drink. After the cessation of drinking, the snakes were thoroughly dried. Narrow strips of "Scotch Tape" were applied to the head in longitudinal strips and in many windings and cross-windings. The entire head was wrapped so that openings were left only at the nostrils. The snakes were then placed in jars (the receptacle being dependent on the size of the snake) and larvae were introduced in water; the amount of fluid used was such as to almost cover the floor of the container. Snakes were confined in contact with larvae in this manner overnight. The following day they were dried, swabbed with 70% alcohol, and placed in clean glass bottles.

This method of restricting drinking was not completely satisfactory, since water tends to loosen the tape, which the snakes may rub off even in perfectly smooth glass containers by rubbing against their own bodies.

Microtechnique

All stages were fixed in hot 70% alcohol. Larvae were stained in alcoholic hydrochloric acid carmine and in Delafield's haematoxylin. The alcoholic carmine stain was found particularly convenient since fixation, staining, and destaining were all carried out in 70% alcohol. Delafield's haematoxylin, however, was useful as an alternative stain to bring out certain details not

visible in carmine preparations. Most noteworthy in this respect were the rudimentary excretory glands and the coelomocytes of the free-living third-stage larvae. Carmine-stained preparations were destained in a solution of one drop of concentrated hydrochloric acid in 100 cc. of 70% alcohol. Haematoxylin-stained specimens were similarly destained in water.

All larval material was cleared in glycerine and mounted in glycerine jelly. Adults were cleared and studied in lactophenol.

Some parasitic third-stage larvae were double embedded in celloidin and paraffin and sectioned at 5 μ. Host tissue, containing encysted larvae, was embedded in paraffin, sectioned at 10 μ, stained in Ehrlich's haematoxylin, and counterstained with eosin.

All drawings were made with the aid of a camera lucida. Phase contrast microscopy was found useful in studying the fine cuticular structures associated with the provisional buccal capsule of the fourth-stage larva. The photograph of a shed provisional buccal capsule (Fig. 8, D) was taken with phase contrast.

Kalicephalus parvus Ortlepp, 1923

The Egg (Fig. 1, A–D)

The eggs of *K. parvus* measure 0.08–0.10 mm. in length by 0.04–0.05 mm. in width. They are transparent, thin-walled, and have slightly dissimilar poles. Eggs are laid in the morula stage.

The First-stage Larva (Fig. 1, E, F) (Measurements in Table I)

In shape the newly hatched larvae are still faintly reminiscent of the embryo in that they are widest at the anterior end and taper from there to the tail. The gradual posterior taper becomes increased in slope near the anus; midway between the anus and the tail, the slope again changes causing the end of the tail to be long and narrow.

The cuticle is fine and, unlike later stages, is not striated.

The musculature is poorly developed; individual cells are indistinguishable, but the narrow muscle nuclei are identifiable.

FIG. 1. Stages in the life history of *Kalicephalus parvus*.
A. Egg, gastrula.
B. Egg, early tadpole.
C. Egg, late tadpole.
D. Egg, embryonated.
E. First-stage larva, lateral view.
F. First-stage larva, ventral view.
G. Second-stage larva, lateral view.
H. Preparasitic third-stage larva, ventral view.
I. Preparasitic third-stage tail, lateral view.
J. Preparasitic third-stage tail, ventral view.
K. Male parasitic third-stage larva, lateral view.
L. Posterior end female parasitic third-stage larva, lateral view.
M. Parasitic third-stage larva, *en face*.
N. Parasitic third-stage larva, cross section at anterior oesophageal region.
O. Parasitic third-stage larva, cross section at level of fourth to sixth intestinal cell.
P. Parasitic third-stage larva, cross section at mid-body.

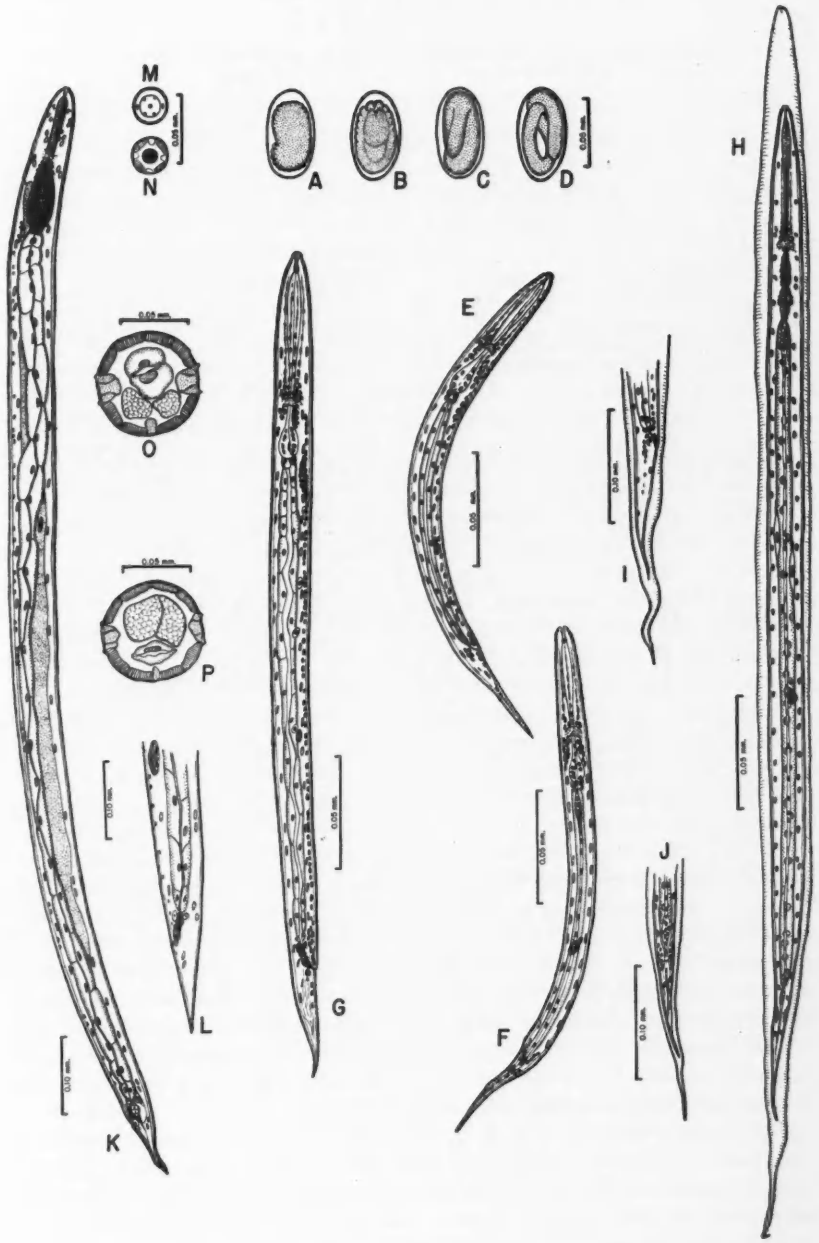
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TABLE I

MEASUREMENTS (MM.) OF FIRST-STAGE, SECOND-STAGE, AND FREE-LIVING THIRD-STAGE LARVAE OF *Kalicephalus parvus*

Item	Stage		
	First	Second	Free-living third
Number of specimens measured	15	15	15
Total length	0.30–0.38	0.46–0.50	0.47–0.59
Maximum width	0.02–0.03	0.02–0.03	0.02–0.03
Length of buccal tube	To 0.01	0.01–0.02	0.01–0.02
Length of oesophagus	0.09–0.13	0.11–0.13	0.12–0.15
Nerve ring from anterior end	0.06–0.08	0.07–0.09	0.09–0.10
Excretory pore from anterior end	0.08–0.10	0.10–0.11	0.10–0.11
Genital primordium from anterior end	0.19–0.22	0.29–0.33	0.27–0.34
Length of tail	0.05–0.07	0.06–0.09	0.05–0.09

The oral opening, surrounded by a small collar, is of slightly greater diameter than the buccal tube, which extends posteriad from it to the oesophagus. The oesophagus is rhabditiform and the isthmus is relatively long, sometimes equalling the corpus in length. There is an intestine of approximately 20 to 22 cells, a delicate, cuticle-lined rectum, and a slightly raised anus.

Surrounding the oesophageal isthmus near its mid-point is the nerve ring, immediately anterior to which are a series of nuclei representing the rudimentary ganglia of the cephalic papillary nerves. The latter, represented by chains of nuclei, extend anteriad from the rudimentary papillary ganglia.

Immediately posterior to the nerve ring, there is visible dorsally a small mass of cells from which evolve the dorsal and subdorsal ganglia of later stages. Ventrally a large ganglionic mass is seen which probably combines the still undifferentiated subventral and posteroventral ganglia. Continuing ventrally and posteriorly, one sees, at the level of the oesophagointestinal valve, the primordium of the retrovesicular ganglion. The lateral ganglia are visible, when the specimen lies in dorsoventral aspect, as two spindle-shaped masses of nuclei extending posteriorly from the nerve ring to the oesophageal bulb. The ventral nerve is represented by a series of nuclei extending along the ventral side of the body from the retrovesicular ganglion, an enlargement in this chain, to the anal ganglion, another enlargement in the chain. Paired, lateral masses of nuclei near the rectum represent the lumbar ganglion.

The excretory system is composed of a minute pore situated ventrally immediately behind the nerve ring and a fine duct that leads posteriorly and is soon lost among the crowded nuclei of this area.

The genital rudiment (Fig. 3) is a small elliptical body ventrally situated just behind the seventh intestinal cell. It is composed of four cells; two polar, crescent-shaped epithelial cells enclose two oval germinal cells. The sexes were not distinguishable.

There is a coelomocyte associated with the genital rudiment. The remaining three coelomocytes, presumably present, could not be identified.

The Second-stage Larva (Fig. 1, G)

Systems not mentioned specifically are as in the preceding stage. Measurements are given in Table I.

This stage is considerably longer than the first. In shape, it tapers gradually both anteriorly and posteriorly from about the level of the fifth intestinal cell.

The cuticle is thin and transparent; striations are fine.

A projection arises from the anterior end of the oesophagus and encloses the base of the buccal tube. The oesophagus has a proportionately shorter isthmus.

The genital primordium (Fig. 3) remains at the level of the seventh intestinal cell. Early in the second stage the genital rudiments of both sexes are similar, but as transition to the third stage begins the male rudiment becomes oval while that of the female remains elliptical. In the male both germinal cells migrate to the narrow posterior end of the rudiment while five or six epithelial cells lie in the bluntly rounded anterior end. In the female the germinal cells remain at opposite poles with most of the epithelial cells lying between them.

All four coelomocytes are now clearly distinguishable, the anterior coelomocyte lying immediately behind the retrovesicular ganglion, and the posterior one lying just anterior to the genital rudiment. The remaining coelomocytes are situated about equidistant from each other and from the first and fourth.

The Free-living Third-stage Larva (Fig. 1, H–J; Fig. 2)

Systems not specifically mentioned are as in the preceding stage. Measurements are given in Table I. The free-living development of *K. parvus* in tap water at room temperature is summarized below.

Hours	Stage	Hours	Stage
0	Morula	48	First stage
6	Early tadpole	72	First ecdysis in progress
9	Late tadpole	96	Second stage
20	Hatching beginning	120	Second ecdysis beginning
24–26	Hatching at its height	144	Third stage

In this stage, the larvae, ensheathed in the cuticle of the preceding stage, appear longer and thinner than those of the second stage, although there is actually little difference in absolute dimensions.

Both the retained exuvium ensheathing the third-stage larva and the cuticle of the larva itself are marked by fine, transverse striations.

The somatic musculature is more strongly developed than in the earlier stages. The rhomboidal shape of the individual cells can be seen. The nuclei are visible as rows of small bodies lying in the four sublateral sectors; they are not as elongate as those of preceding stages.

The mouth has been considerably reduced. The oesophagus is long and slim, the bulb is no longer prominent. In stained preparations, the intestinal nuclei, 32 in number, are seen to be large and circular or oval.

There are six papillary nerves (PN) (Fig. 2) represented in the larva by chains of nuclei arising from nuclear masses immediately adjacent to the anterior face of the nerve ring. The chains proper, two subdorsal, two subventral, and two lateral, are the primordia of the adult cephalic papillary

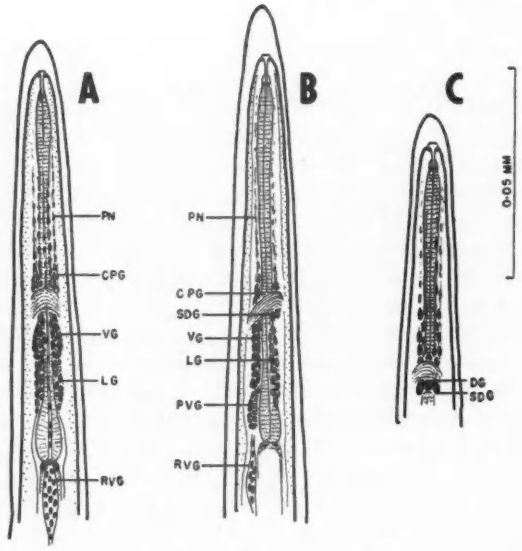

FIG. 2. Anterior end of preparasitic third stage of *Kalicephalus parvus*.
A. Ventral view.
B. Lateral view.
C. Dorsal view.
CPG—cephalic papillary ganglion; DG—dorsal ganglion; LG—lateral ganglion; PN—papillary nerve; PVG—posteroventral ganglion; RVG—retrovesicular ganglion; SDG—subdorsal ganglion; VG—ventral ganglion.

nerves, while the denser, proximal nuclear masses represent the future cephalic papillary ganglia (CPG). The nerve ring is indicated by a relatively colorless band encircling the oesophagus at its isthmus. Immediately posterior and adjacent to the nerve ring are the primordia of several cephalic ganglia. Viewed dorsally, the small dorsal (DG) and subdorsal ganglia (SDG) are represented by a trilobed, closely associated nuclear grouping. The two outer lobes, the subdorsal ganglia, enclose the inner dorsal ganglion. In lateral aspect, the dorsal and subdorsal ganglia are visible, encroaching upon the nerve ring to the dorsal side of the oesophagus; posterior and lateral to these a large nucleate mass, one of a pair of lateral ganglia (LG) extends to the oesophageal bulb. Ventrally three ganglia can be seen in sequence extending posteriorly from the nerve ring. These are: (1) one of the large, paired ventral ganglia (VG); (2) a nucleate mass surrounding the excretory duct and termed the posteroventral ganglion (PVG) by Alicata (1), but considered to be the primordium of the excretory complex by Looss (11); and (3) in the most posterior position, i.e., just behind the oesophageal bulb, the retrovesicular ganglion (RVG) (ventral cephalic ganglion of Looss).

The ventral nerve is represented by a chain of nuclei with sharply defined longitudinal margins which extends from close to the anterior end of the oesophagus into the retrovesicular ganglion, and from there continues posterior

to another enlargement, the anal ganglion. From this, after passing around the anus, it again extends posteriad, gradually diminishes in width, and terminates in the tail close to the posterior end. The paired lumbar ganglia lie laterally at a level just behind the anal ganglion.

The excretory pore is as in the preceding stages. However, the primordia of the excretory glands are now visible, lying between the intestine and the body wall a short distance anterior to the genital primordium. The rudimentary excretory glands are spindle-shaped; their nuclei are long and narrow. Attenuated anterior prolongations of these cells are sometimes visible.

The sexes are distinguishable with certainty on the basis of the genital rudiment (Fig. 3). In the female, it is elliptical, while in the male it is oval or comma-shaped. The blunt end of the male genital rudiment lies toward the anterior. There are approximately eight epithelial cells and two germinal cells, the latter lying in the posterior part of the rudiment.

The female rudiment is composed of two germinal cells, one of which lies at or close to each end, and eight epithelial cells. A pair of these are polar or tend to encroach on the polar position, while three pairs are symmetrically arranged along the middle of the rudiment body.

The Parasitic Third-stage Larva (Fig. 1, K–P; 4; 5; 8, A, B)

The larvae introduced per os remain ensheathed and free in the stomach until about the 10th day. In measurements and morphology, these are similar to the free-living larvae.

By the 14th day, the larvae have penetrated the stomach wall where they encyst in the mucosa. Encysted parasitic third-stage *K. parvus* larvae were found only in the stomach. The cyst when first formed contains a clear, slightly amber-colored liquid and has an elastic wall. As the larva becomes older, there is often marked haemorrhage in the cyst area and the cysts themselves sometimes contain blood. In heavy infections, the stomach, on macroscopic examination, shows haemorrhagic areas several millimeters in diameter (Fig. 8, A).

Newly Encysted Larvae (14–18 days)

These are exsheathed, but resemble the free-living third stage in morphology. They are longer, varying from 0.60–0.98 mm. in length, and the only marked differences occur in the excretory and reproductive systems. The excretory glands are no longer rudimentary. They are granular and have very large elliptical nuclei.

The genital primordium in the female is now located close to the tail and its shape is a long ellipse. The more anteriorly situated male rudiment, oval in shape, is essentially as in the preceding stage. The coelomocytes are seen as long, spindle-shaped cells with a large central nucleus. One of these is usually associated with the genital rudiment.

The tail in both sexes is proportionately shorter than in the free-living third stage, but as yet both sexes have the same over-all tail shape.

Typical Encysted Larvae (35–109 days)

After 35 days gross sexual dimorphism is evident. The female has a long, evenly tapering tail, while in the male the taper is abrupt (Fig. 1, K, L). The fine anatomy is now typical of the parasitic third stage (Fig. 1, K–P). Prior to this, a transition from the free-living stage is still in progress; in most systems a period of little or no change now ensues. During this period only the genital rudiment of the male undergoes a marked morphological alteration, but this is slow. Toward the end of the period the beginnings of the formation of the provisional buccal capsule are seen.

There is little published information on the anatomy of the parasitic third stage of nematodes and since an abundance of material was available, considerable study was given to this stage. Measurements of parasitic third-stage larvae are given in Table II.

The thin, transparent cuticle is not striated. It is longitudinally ridged along the sides of the lateral lines.

The musculature is meromyarian and platymyarian. In cross section taken at mid-body, there are two muscle cells visible in each of the four body sectors (Fig. 1, P). The muscle cells are rhomboid. It is difficult to count the nuclei of the somatic musculature, but of varying totals between 63 and 70, the author has most confidence in counts of 68 nuclei.

The longitudinal chords are simple. The lateral chords each contain three rows of nuclei, the longitudinal walls between the three rows being distinct. Transverse walls between nuclei of the same row were not seen.

A simple mouth and buccal tube are present. The oesophagus has a large oval bulb; there is no visible demarcation of isthmus and corpus. The paired subventral oesophageal glands extend from close to the posterior end of the oesophagus to a point a short distance anterior to the nerve ring. The single dorsal gland extends anteriorly to the posterior end of the buccal tube. In the base of each sector of the bulb a large nucleus is visible; that of the dorsal sector is the largest. The intestine is composed of 32 cells. The rectum, a delicate, cuticularized tube, leads from a well-developed intestino-rectal sphincter to the anus.

Laterally, close to the anterior end, the amphidial pores are seen at the level of the posterior end of the buccal tube. From each pore the amphidial canal extends posteriad to terminate in an amphidial pouch. The cephalic papillary ganglia are located a short distance anterior to the nerve ring. They lie close to the oesophagus. The nerve ring is prominent and in its immediate vicinity the several main ganglia of the central nervous system are visible. The lateral ganglia appear as pendulous clusters of cells closely applied to the oesophagus. The single dorsal and the paired subdorsal ganglia are small. The former lies close to the nerve ring and the latter lie further posterior. The subventral ganglia occur just anterior to the anterior ends of the excretory glands. The retrovesicular ganglion lies ventral to the first intestinal cell. The ventral nerve continues to be marked by a series of nuclei; these, however, are now spaced at long intervals. The anal ganglion

TABLE II

MEASUREMENTS (MM.) OF PARASITIC THIRD-STAGE LARVAE OF *K. parvus* AT VARIOUS TIME INTERVALS AFTER INFECTION

Character measured	Number of days in host														
	27		36					92		109		130			
Females															
Total length	1.32	1.56	1.83	1.79	1.83	1.74	1.85	1.95	2.13	2.03	2.10	2.18	2.37	2.43	
Maximum width	0.06	0.06	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.11	0.12	0.09	0.11	0.12	
Length of buccal tube	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	
Nerve ring from anterior	0.11	0.09	0.12	0.12	0.13	0.12	0.14	0.12	0.12	0.13	0.12	0.11	0.13	0.12	
Excretory pore anterior	0.14	0.15	0.16	0.16	0.16	0.16	0.17	0.16	0.18	0.18	0.16	0.17	0.18	0.20	
Length of oesophagus	0.20	0.20	0.23	0.21	0.23	0.23	0.23	0.24	0.23	0.26	0.26	0.26	0.26	0.29	
Width of oesophagus	0.03	0.03	0.03	0.03	0.05	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.05	0.05	
Length of tail	0.08	0.10	0.09	0.07	0.09	0.08	0.09	0.11	0.12	0.09	0.11	0.10	0.12	0.10	
Genital primordium from anterior	1.05	1.25	1.50	1.46	1.50	1.43	1.49	1.58	1.59	1.64	1.71	1.71	1.92	2.01	
Males															
Total length	1.43		1.50	1.67	1.62	1.76	1.65	1.83	1.80	1.91	1.89	2.07	2.22	2.16	
Maximum width	0.06		0.06	0.06	0.08	0.09	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.09	
Length of buccal tube	0.01		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	
Nerve ring from anterior	0.10		0.12	0.12	0.12	0.11	0.11	0.11	0.12	0.11	0.11	0.13	0.13	0.12	
Excretory pore anterior	0.13		0.17	0.16	—	0.17	0.15	0.16	0.17	0.18	0.16	0.18	0.17	0.18	
Length of oesophagus	0.18		0.21	0.21	0.23	0.24	0.21	0.23	0.24	0.23	0.24	0.24	0.24	0.24	
Width of oesophagus	0.03		0.03	0.03	0.03	0.05	0.03	0.05	0.05	0.04	0.05	0.05	0.05	0.05	
Length of tail	0.06		0.06	0.06	0.07	0.07	0.08	0.09	0.09	0.09	0.09	0.11	0.11	0.09	
Genital primordium from anterior	1.87		1.96	1.02	0.93	1.05	0.98	1.08	1.11	1.16	1.11	1.23	1.32	1.29	

(Fig. 4) is divided into two parts, the first, the anteroanal (Aa G), lying ventrally at the level of the intestino-rectal sphincter, and the paired posteroanal ganglia (Pa G) lying one on either side of the rectum shortly before the anus. The latter ganglia form a compact, intensely staining, triangular body. Dorsal and lateral to each posteroanal ganglion is a larger, but more loosely associated, triangular ganglion (Lu Ga_1). The latter is paired and represents the lumbar ganglion in part. Posteriorly each is connected with a second lumbar division (Lu Ga_2), which innervates the phasmids. There is a ventral connection to the posteroanal ganglion and anteriorly the tapering out represents the connection of the ganglion with the nerves of the lateral chords. The phasmids are seen laterally about midway between the tip of the tail and the anus.

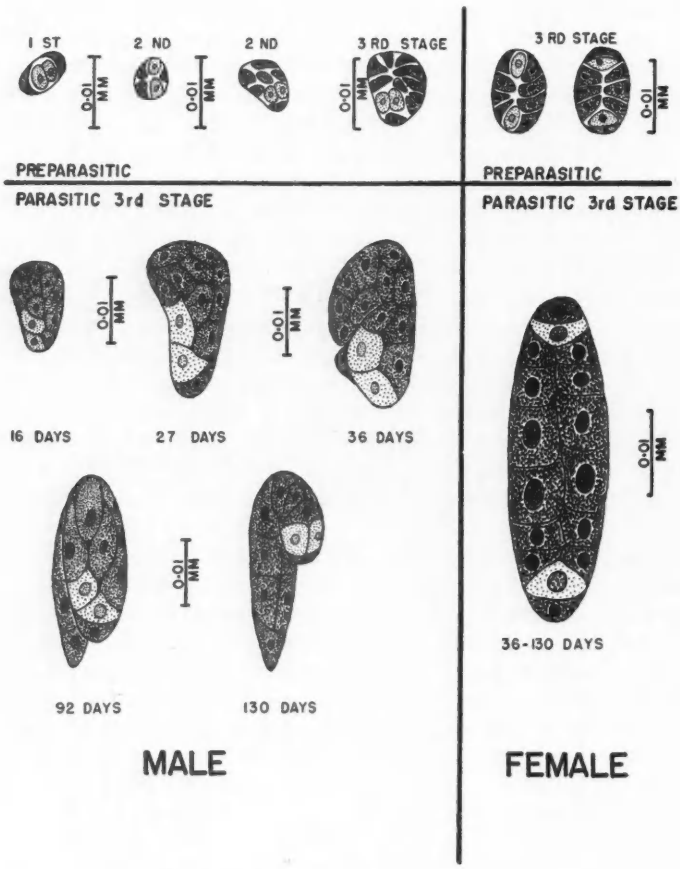

FIG. 3. The development of the genital primordium in *Kalicephalus parvus*.

The excretory pore lies ventrally just anterior to the level of the maximum width of the oesophageal bulb. From it, the excretory duct leads to an excretory sinus enclosed between the rounded anterior ends of the paired excretory glands. The latter, lying ventrolaterally to the oesophagus, extend posteriorly. In the area of the fifth to sixth intestinal cell, the excretory cells bend over the intestine and come to lie side by side dorsolaterally to the intestine. The nuclei are found in the area in which the crossing over occurs. Each gland possesses a large, elongate, deep-staining nucleus. The excretory glands are unequal in length; at their posterior ends they diminish in size and their ends are relatively sharply drawn. The longer excretory gland terminates in the region of the fourth intestinal cell from the rear.

During the first 27 days of parasitic life, the anterior end of the male genital rudiment is gradually beginning to bulge to one side (Fig. 3). After 36 days, the anterior bulge has elongated and is bent back upon the posterior part of the rudiment. The latter has become enlarged. The germinal cells are gradually shifting into a posterior polar position and bear a single-celled epithelial cap. The epithelial cells number 12 to 14.

Gradually the tail-like, posteriorly bending portion elongates until it is as long as the wider portion containing the germ cells. It is now obvious that the structure is reversing itself and retaining the germinal cells in what will become the anterior position.

By the 130th day in the host, some rudiments have almost completed the reversal. Throughout this reorientation, the number of epithelial cells remains unchanged.

In the female, there is an increase in the number and size of the cells, with a consequent elongation of the rudiment. When the 19-celled condition is attained, development becomes static. There are at this time two germinal cells capped by two polar epithelial cells. The germinal cells are separated by 12 paired epithelial cells beneath which three single epithelial cells lie along the axis of the rudiment. This condition was observed through the 130th day.

Immediately posterior to the intestino-anal sphincter (Sph) and subventral to the rectum, two epithelial cells (Ep *a v*) partially surround the origin of the rectum (Fig. 4). From these, three pairs of epithelial cells (Ep *p*) extend posterially along the course of the rectum and lie lateroventral to it. Viewed laterally, these are small flattened cells lying in close association. Viewed ventrally, they form a high arc, the open ends of which lie toward the anus. The closed end is formed by the ventral anterior pair of epithelial cells (Ep *a v*).

Dorsal to the origin of the rectum, another pair of epithelial cells (Ep *a d*) complete the encirclement of the origin of the rectum. Posterior and partially dorsal to these lie the three anterior cells (Sp P_1, Sp $P_{5a, 5b}$) of the spicular primordium, which is a grooved ovoid mass with a posterior projection when viewed in dorsoventral optical section. The three cells already mentioned form the anterior broad end of the oval. Next, posteriorly, two pairs of cells (Sp $P_{6a, 6b}$; Sp $P_{7a, 7b}$) along each lateral margin form the long sides of the

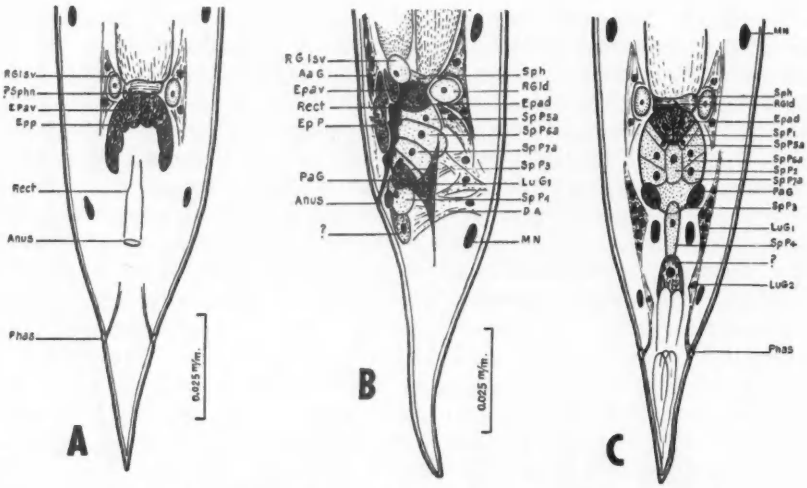

FIG. 4. Tail of male parasitic third-stage larva of *Kalicephalus parvus*.
A. Ventral view of structures ventral to rectum.
B. Lateral view.
C. Ventral view of structures dorsal to the rectum.

Aa G—anteroanal ganglion; AG—anal ganglion; DA—depressor ani; Ep—epithelial cell; Ep *a d*—anterior dorsal epithelial cell; Ep *a v*—anterior ventral epithelial cell; Ep *p*—posterior epithelial cell; Lu G_1—first division of lumbar ganglion; Lu G_2—second division of lumbar ganglion; MN—muscle nucleus; Phas—phasmid; Pa G—posteroanal ganglion; Rect—rectum; R Gl *d*—dorsal rectal gland; R Gl *sv*—subventral rectal gland; Sph—sphincter; ?Sph N—?sphincter nucleus; Sp P_{1-4}—single cells of the spicular primordium; Sp P_{5a-7a}—paired cells of the spicular primordium; ?—cell of unknown nature.

oval and are separated medially by an elongate rectangular cell (Sp P_2). Posterior to these, a single large cell (Sp P_3) forms the narrow part of the oval. Finally, originating ventral to the latter and extending posteriorly just dorsal to the distal end portion of the rectum, is the single elongate cell (Sp P_4) forming the posterior projection of the spicular rudiment.

Late Parasitic Third-stage Larva (Fig. 5)

These larvae, now approximately 2.00 mm. in length, occur in more obvious cysts. With the naked eye, the latter appear as small, glistening elevations. Observed with a hand lens, the cyst itself presents the appearance of a raised, shiny, translucent nodule in which the ivory-colored larvae are readily visible. The largest cysts measure approximately 2.00 by 1.50 mm.

As already noted, after the larvae have been approximately 36 days in the host, there ensues a long static period. In this period, besides changes in the male genital rudiment, there is gradual increase in over-all size and in the size of most organs, but these in general maintain the same structure as found in the 36 day parasitic larvae.

By the 109th day, however, transition to the fourth stage is beginning and the formation of the provisional buccal capsule merits discussion. At first,

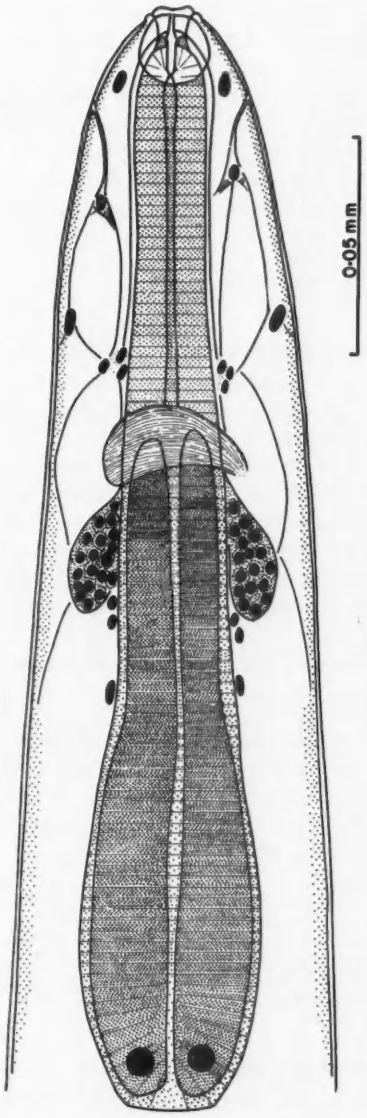

FIG. 5. Anterior end of a late parasitic third-stage larva of *Kalicephalus parvus*.

two vacuolations are seen, one dorsal and one ventral to the anterior end of the buccal tube; the dorsoventral diameter across both vacuolations at their base is about 0.02 mm. At this time, the teeth of the provisional buccal capsule begin their development at the anterior end of the oesophagus and the cuticle is just starting to loosen anteriorly. By the 130th day, each vacuolation extends posteriorly from the end of the buccal tube and encompasses part of the anterior rounded-off tip of the oesophagus. The latter appears to be undergoing a change in texture in the area where it is enclosed by the vacuolations. This probably represents the onset of oesophageal dissolution where it extends into the area which will become the new buccal capsule.

Fourth-stage Larva

No fourth-stage larvae were recovered.

Fifth-stage (Adult)

K. parvus eggs were collected from a gopher snake, *Pituophis catenifer affinis*, 115 days after experimental infection and thus adult worms were present at this time. This snake was killed 461 days after infection and four male and three female *K. parvus* were recovered.

The prepatent period of 115 days as compared to the previously stated condition where *K. parvus* had not progressed further than the third stage in 130 days may be attributable to a host difference. The latter time is based on studies in which garter snakes and Dekay's snakes were used.

A morphological description of the adults is omitted since these are adequately described by Harwood ((9) (*K. tennesseensis* = *K. parvus*)).

Kalicephalus agkistrodontis Harwood, 1932

The Egg and Preparasitic Larvae (Fig. 6, A–G)

The egg and free-living larvae of *K. agkistrodontis* are essentially the same as those of *K. parvus* and therefore are not discussed in detail. The measurements of the three preparasitic stages are given in Table III. The eggs of *K. agkistrodontis* measure 0.07–0.08 by 0.04–0.05 and development in tap water at room temperature is outlined below.

Hours	Stage	Hours	Stage
0	32-celled	24	Hatching almost complete
6–7	Gastrula	48	Some first ecdysis; mostly second stage
12	Early tadpole		
16	First fully developed embryos	72	All in second stage
19	Fully developed embryos; hatching beginning	96	Transition to third stage
		120	All in third stage
20–22	Hatching at height		

The Parasitic Stages of K. agkistrodontis

K. agkistrodontis differs markedly from *K. parvus* in its parasitic life history. The differences are two, the first being in time of development. *K. agkistrodontis* was found in the adult or fifth stage, although immature, in 23 days. Secondly, *K. agkistrodontis* does not encyst. All parasitic stages occur in the lumen of the digestive tract.

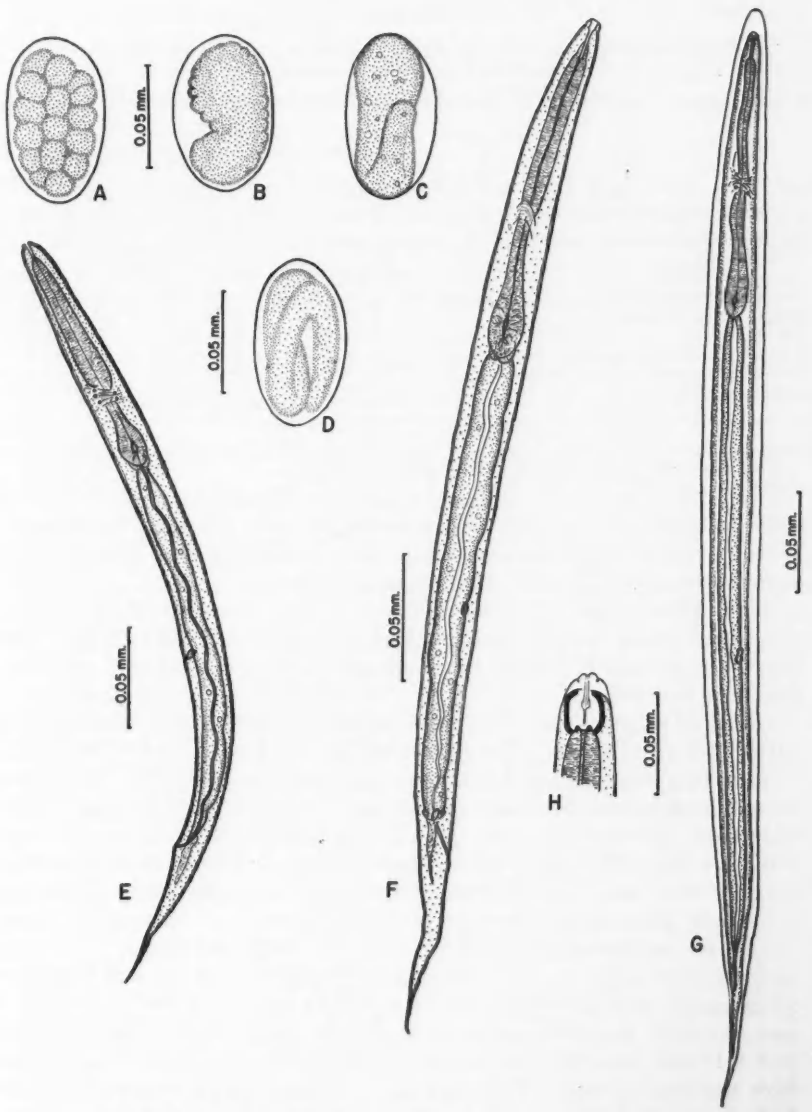

FIG. 6. Stages in the life history of *Kalicephalus agkistrodontis*.
A. Egg, 32-celled.
B. Egg, gastrula.
C. Egg, tadpole.
D. Egg, embryonated.
E. First-stage larva, lateral view.
F. Second-stage larva, lateral view.
G. Third-stage larva, ventral view.
H. Formation of provisional buccal capsule.

TABLE III

MEASUREMENTS (MM.) OF FIRST-STAGE, SECOND-STAGE, AND PREPARASITIC THIRD-STAGE LARVAE OF *K. agkistrodontis*

Item	Stage		
	First	Second	Preparasitic third
Number of specimens measured	14	19	18
Total length	0.31–0.36	0.40–0.49	0.49–0.57
Maximum width	0.01–0.02	0.02–0.03	0.02–0.03
Length of buccal tube	To 0.01	0.01–0.02	0.01–0.02
Length of oesophagus	0.09–0.11	0.11–0.12	0.11–0.14
Nerve ring from anterior end	0.07–0.08	0.08–0.09	0.08–0.09
Excretory pore from anterior end	0.08–0.09	0.09–0.10	0.09–0.12
Genital primordium from anterior end	0.19–0.21	0.23–0.28	0.29–0.34
Length of tail	0.06–0.07	0.06–0.08	0.04–0.07

The Parasitic Third-stage Larva (Fig. 6, H)

Two days after oral administration of infective larvae, numerous individuals, still ensheathed, were recovered from the lumen of the stomach. These resembled the preparasitic larvae in all observed particulars, including over-all dimensions and size of individual structures.

By the 17th day the larvae, free in the duodenum, have undergone considerable development. One specimen had attained the fourth stage, most individuals were in a state of transition, and a few still displayed the third-stage characteristics.

The third-stage larvae at 17 days measure 0.86–1.14 mm. in length by 0.03–0.06 mm. in width. The genital rudiment has enlarged and in females it is a spindle-shaped mass of cells. As yet, it is not connected with the vulva, but the primordium of the latter is seen as several cells proliferating from the body wall. In the males, the genital rudiment has bent back upon itself with both sides of the bend about equal in length. The genital primordium lies 0.53–0.69 mm. from the anterior end. The most slowly developing individuals show no development of the buccal capsule whereas the larvae which have progressed furthest have already a completely formed provisional buccal capsule (Fig. 6, H). The buccal capsule in the specimens studied varied in size from 0.06–0.07 mm. by 0.05–0.08 mm. The old buccal tube is seen attached to the loosened cuticle. The nerve ring is located about 0.13–0.15 mm. from the anterior end; the excretory pore about 0.15–0.16 mm. from the anterior end. The oesophagus, proportionately shorter and more bulbed, is 0.15–0.20 mm. long. The anus is situated 0.08–0.09 mm. from the posterior.

The Fourth-stage Larva

In addition to a single individual that was found in the small intestine after 17 days and which had apparently just entered the fourth stage, two well-developed fourth-stage larvae were found after 23 days. One of these was

recovered from the duodenum and the other from the stomach. These larvae possess a large provisional buccal capsule behind which the developing adult buccal capsule is in evidence. In this and other characters the fourth stage larvae of *K. agkistrodontis* resemble those of *K. rectiphilus.*

Fifth-stage (Adult) (Table IV)

Adults, not yet mature, were recovered after 23 and 27 days. In these the reproductive system was not completely developed. On the 58th day two mature, adult specimens were recovered from an experimentally infected snake. The female was gravid; her eggs measured 0.08 by 0.05 mm.

A morphological description of the adults is available in Harwood (8).

TABLE IV

MEASUREMENTS (MM.) OF FIFTH-STAGE *K. agkistrodontis* AT VARIOUS TIMES OF DEVELOPMENT IN THE HOST

Character measured	Subadult						Adult 58 days
	23 days		27 days		29 days		
Females							
Total length	2.64	3.26	4.11	5.12	4.29	3.54	8.57
Maximum width	0.14	0.17	0.21	0.23	0.29	0.23	0.42
Head diameter	0.11	0.14	0.15	0.17	0.21	0.18	0.20
Depth buccal capsule	0.08	0.08	0.11	0.09	0.12	0.11	0.12
Nerve ring from anterior	0.17	0.18	0.18	0.18	0.23	0.20	0.21
Excretory pore anterior	0.26	0.24	0.32	0.26	0.30	0.28	0.30
Length of oesophagus	0.23	0.24	0.26	0.24	0.27	0.27	0.26
Width of oesophagus	0.09	0.09	0.11	0.12	0.15	0.15	0.15
Vulva from anterior	1.73	2.21	2.66	3.23	2.90	3.30	5.40
Vulvar ratio	1.9 : 1	2.1 : 1	1.8 : 1	1.7 : 1	2.1 : 1	1.8 : 1	1.7 : 1
Length of tail	0.12	0.11	0.12	0.14	0.14	0.12	0.30
Males							
Total length	2.27	2.48	4.05		2.75	3.83	6.78
Maximum width	0.14	0.14	0.18		0.18	0.23	0.32
Head diameter	0.14	0.14	0.14		0.15	0.15	0.18
Depth buccal capsule	0.08	0.08	0.09		0.09	0.09	0.11
Nerve ring from anterior	0.15	0.17	0.15		0.17	0.17	0.20
Excretory pore anterior	0.23	0.24	0.21		0.27	0.26	0.23
Length of oesophagus	0.20	0.23	0.21		0.24	0.23	0.24
Width of oesophagus	0.09	0.08	0.11		0.12	0.11	0.12
Length of spicule	*	0.40†	0.45		0.51	0.48	0.48

**Spicules not completely formed.*

†*Measured approximately; spicules incompletely sclerotized, sinuous, and difficult to measure exactly.*

TABLE V

MEASUREMENTS (MM.) OF FIRST-STAGE, SECOND-STAGE, AND PREPARASITIC THIRD-STAGE OF *K. rectiphilus*

Item	Stage		
	First	Second	Preparasitic third
Number of specimens measured	5	5	10
Total length	0.31–0.34	0.41–0.56	0.52–0.70
Maximum width	0.01–0.02	0.02–0.03	0.02–0.03
Length of buccal tube	To 0.01	0.01–0.02	0.01–0.02
Length of oesophagus	0.09–0.10	0.11–0.13	0.12–0.19
Nerve ring from anterior end	0.07–0.08	0.08–0.09	0.08–0.11
Excretory pore from anterior end	0.08–0.09	0.09–0.10	0.09–0.12
Genital primordium from anterior end	0.18–0.19	0.30–0.31	0.30–0.37
Length of tail	0.05–0.06	0.06–0.08	0.04–0.10

Kalicephalus rectiphilus Harwood, 1932

Preparasitic Stages

The egg and the free-living larvae of *K. rectiphilus* are essentially like those of *K. parvus* in development, measurements, and structure. Therefore, measurements (Table V) and development (below) are reported in tabular form only.

Parasitic Stages

This species differs from *K. agkistrodontis* in that the early parasitic stages are encysted. It differs from *K. parvus*, in which histotropic stages also occur, in the site of encystation, the intestine, whereas *K. parvus* encysts in the stomach.

Hours	Stage	Hours	Stage
0	Morula	48	Some second-stage larvae; most larvae in molt
6–9	Tadpole stage		
18	Embryos fully developed	60	Majority second-stage larvae
20	Hatching beginning	72	Some third-stage larvae
24	Hatching at its height	96	Majority third-stage larvae
		108	All third-stage larvae

Parasitic Third-stage Larvae (Table VI)

Parasitic third-stage larvae were recovered from cysts in the mucosa of the posterior part of the duodenum at 26, 28, and 33 days after infection. Additional material was obtained for which the time of development was not known. The following description is based on the total material consisting of 26 specimens.

The parasitic third-stage larvae of *K. rectiphilus* can be morphologically differentiated from those of *K. parvus* as follows: (1) the female genital rudiment is situated nearer the mid-body; (2) the excretory glands are slimmer and shorter; (3) the intestine is composed of fewer cells, 30–32; (4) the retrovesicular ganglion is more posterior, at the level of the third to fourth intestinal cell.

TABLE VI

MEASUREMENTS (MM.) OF THE PARASITIC THIRD-STAGE LARVAE OF *K. rectiphilus*

Item	Male	Female
Number of specimens measured	17	9
Total length	2.00–3.06	2.42–3.04
Maximum width	0.09–0.12	0.09–0.12
Length of oesophagus	0.19–0.24	0.21–0.25
Width of oesophagus	0.03–0.04	0.03–0.04
Nerve ring from anterior end	0.10–0.13	0.11–0.15
Excretory pore from anterior end	0.16–0.23	0.19–0.22
Genital primordium from anterior end	1.11–1.94	1.48–1.88
Length of tail	0.06–0.09	0.11–0.14

The genital rudiment (Fig. 7, F–I) is located at the level of the 9th to 10th intestinal cell. In this character *K. rectiphilus* differs markedly from *K. parvus* where the female genital rudiment is located near the tail (13th intestinal cell). In *K. rectiphilus* the genital rudiments of each sex structurally resemble those of like sex of *K. parvus* at the same degree of development. Thus, in the male 26–33 days after infection, the shape of the genital primordium, the number of contained cells, and the arrangement of cells is similar for both species. In *K. rectiphilus* there are 14 cells, 12 epithelial cells and two germinal cells (Fig. 7, G–I). The germinal cells are situated near the posterior end of the rudiment, one epithelial cell lying behind them.

Observations on the spicular primordium in the parasitic third-stage larvae of *K. rectiphilus* are virtually in total agreement with those made on *K. parvus*. Only the existence of the most anterior single cell of the spicular primordium of *K. parvus* (Fig. 3, Sp P_1) could not be confirmed.

Fourth-stage Larva (Fig. 7, A, B; Fig. 8, B)

Two female fourth-stage larvae were recovered at 33 days, together with one third-stage female and nine subadults. All were encysted in the mucosa of the posterior part of the duodenum.

In the fourth-stage larvae, the transition to the adult was advanced. The definitive buccal capsule was well differentiated, the provisional buccal capsule of one specimen being partially detached and both specimens showing a loosening of the cuticle. The development of the reproductive system had progressed as far as that of the female subadults recovered at the same time.

The two female fourth-stage larvae measure 3.82 and 4.00 mm. in length and 0.18 mm. in width. The provisional buccal capsule is 0.04 mm. high and and 0.05 mm. in greatest diameter. In its base are three teeth which connect to the oesophagus by a cuticularized duct. Two small pits, whose function is unknown, open into the anterior part of the provisional buccal capsule. The additional measurements taken are as follows: depth of definitive buccal

capsule, 0.10 mm.; oesophagus, 0.23 and 0.24 mm. in length; oesophagus, 0.07 and 0.10 mm. in maximum width; nerve ring and excretory pore 0.16 and 0.25 mm. from the anterior end respectively. The vulva divides the body in the ratio 1.7 : 1. The tail is 0.22 mm. long.

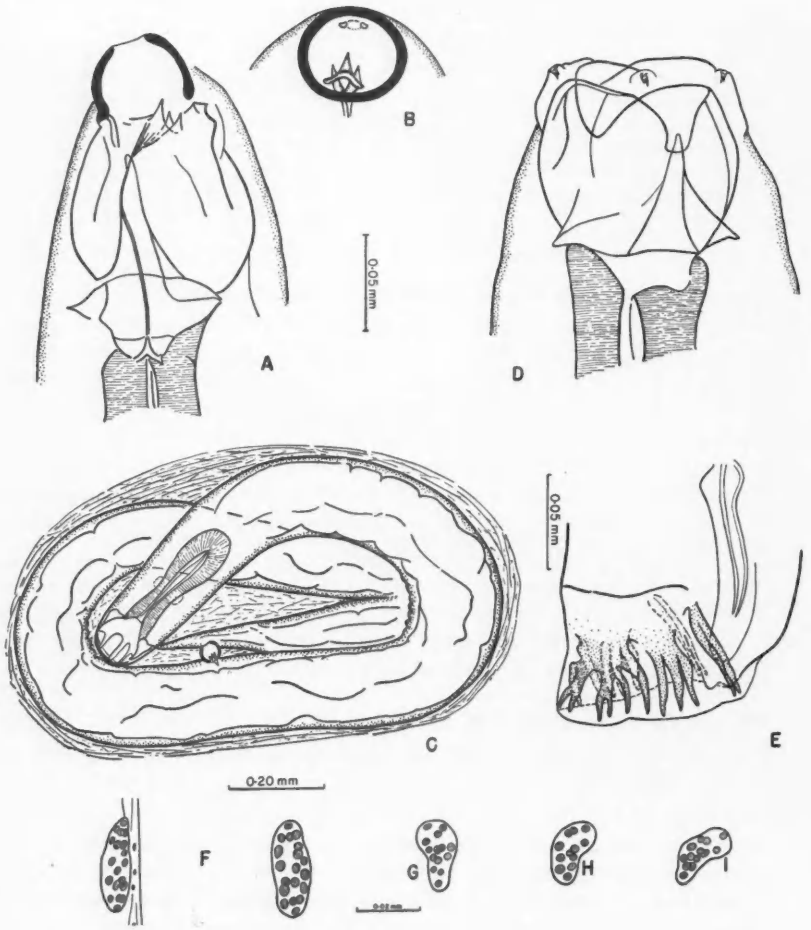

FIG. 7. Parasitic stages of *Kalicephalus rectiphilus*.

A. Fourth stage anterior end showing formation of definitive buccal capsule, dorsal view.
B. Provisional buccal capsule.
C. Encysted subadult; cast provisional buccal capsule included in cyst.
D. Subadult anterior end, dorsal view.
E. Subadult, developing bursa.
F. Female genital rudiment of parasitic third stage (1) lateral, (2) ventral.
G.—I. Male genital rudiment of parasitic third stage.

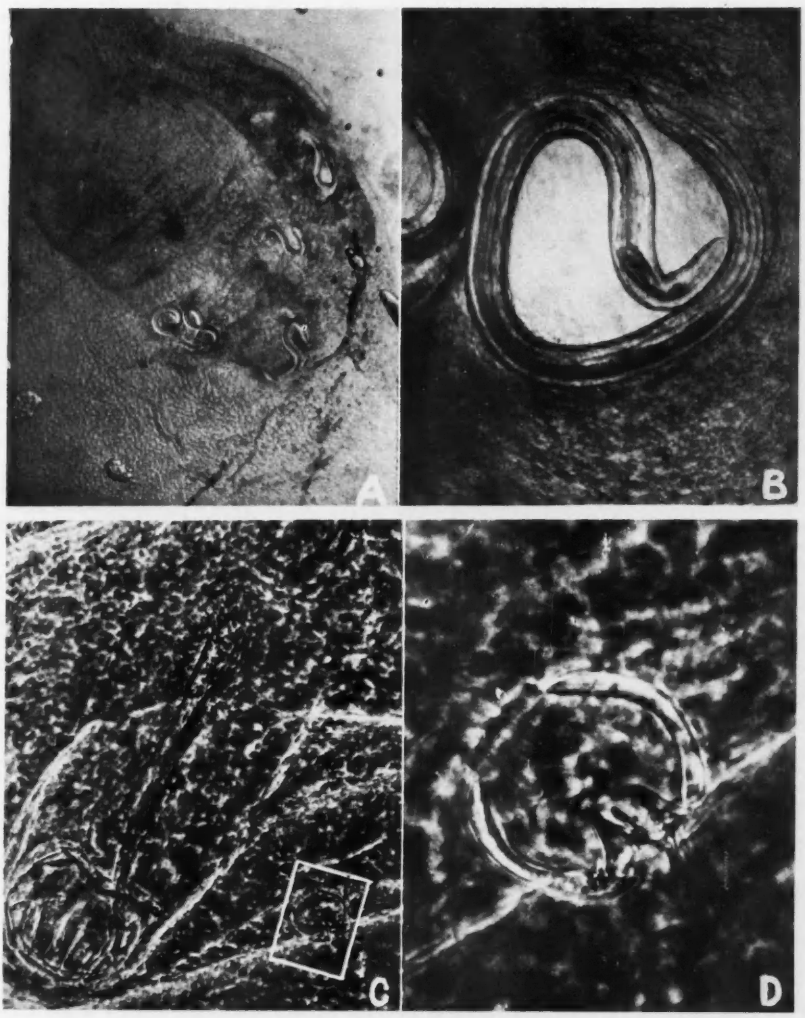

FIG. 8. A. Haemorrhagic stomach showing encysted third-stage *Kalicephalus parvus*. × 13
B. An enlargement of a cyst of 8A—male larva in cyst. × 108
C. Encysted subadult of *Kalicephalus rectiphilus*. Same individual as FIG. 7C. Cast provisional buccal capsule included within cyst. × 200
D. Enlargement of cast provisional buccal capsule within cyst shown in 8C. × 800

Fifth Stage (Adult)

Subadults (Fig. 7, C–E; Fig. 8, A) are delicate nematodes 2.94–4.05 mm. in length and 0.16–0.19 mm. in width (Table VII). All systems are feebly developed as compared to those of the adult and little progress beyond the structural characteristics of the most advanced members of the preceding stage has occurred.

The buccal capsule is not completely cuticularized; nevertheless its kalicephalid character is apparent. It is 0.10–0.13 mm. deep.

The reproductive system is just beginning to display its adult characteristics. All the various structures are present, but only in a very underdeveloped state. The bursa of the male is short and stumpy. Although all rays are present, they have not reached adult proportions. In some specimens the spicules are indefinite, while in others cuticularization has progressed to a point where the definitive spicular dimensions are visible but hardening is incomplete. In the latter, the spicules measure 0.22–0.25 mm. in length. The testis and tubes of the male reproductive system are all indicated in the rudimentary system seen, but these are small in size and poorly differentiated. The anterior tip of the testis remains in the posterior two-thirds of the body.

In the female, the opening of the vulva is indicated but the ovejector is not as yet heavily muscularized; its general shape and proportions are merely outlined. The uteri are present as wide tubes and each of the solid ovaries extends only a short distance from its origin before bending back on itself and extending to the area of the uterus.

Forty-three days after infection, adult *K. rectiphilus* were recovered from the rectum of an experimentally infected snake. Only males were recovered and therefore information on the prepatent period in this species cannot be presented. Eggs were recovered from the faeces of an experimentally infected snake 92 days after infection, but it is doubtful that this represents the earliest date on which eggs could have been recovered. This snake was killed after 354 days and two female and three male *K. rectiphilus* were recovered. A morphological description of the adult of *K. rectiphilus* is given by Harwood (8).

TABLE VII

MEASUREMENTS (MM.) OF *K. rectiphilus* SUBADULTS (33 DAYS IN HOST)

Character measured	Males					Females	
Total length	3.53	3.68	3.09	2.94	3.53	4.05	3.90
Maximum width	0.18	0.16	0.19	0.18	0.16	0.19	0.18
Head diameter	0.14	0.16	0.15	0.14	0.16	0.18	0.17
Depth buccal capsule	0.12	0.12	0.10	0.12	0.10	0.13	0.13
Nerve ring from anterior	0.20	0.21	0.18	0.20	0.20	0.23	0.26
Excretory pore from anterior	0.30	0.28	0.28	0.26	0.28	0.30	—
Length of oesophagus	0.21	0.22	0.24	0.21	0.25	0.23	0.24
Width of oesophagus	0.07	0.10	0.11	0.10	0.11	0.11	0.11
Length of spicule	0.25	0.23	—*	0.22	—*		
Length of tail						0.14	0.20

**Not cuticularized.*

Experiments on Skin Penetration

The results of these experiments indicate that skin penetration may occur. Thirteen snakes were exposed to larvae as described in the section on materials and methods. Of the 13 snakes used, 10 were negative on post-mortem examination while three were positive. Of the three positives, one was exposed to infective larvae in water, the prevention of drinking was successful and 40% of the larvae were recovered as later stages in the host. In the case of the remaining infected snakes, one had shed its tape and therefore results obtained (8% recovery) from it are of doubtful value. On the third snake, the tape preventing drinking held and 2% of the larvae placed in contact with the snake were subsequently recovered as parasites. All other snakes used in these experiments were negative in spite of their exposure to greater numbers of vigorous larvae.

The author is uncertain whether oral taping as described can be said to definitely rule out infection per os. While it certainly did prevent drinking whenever it held, it seems possible that, after the tape had become wet and lost some of its adhesive properties, the larvae could have entered the mouth by crawling between the skin and tape and finally between the closed lips.

The only consistent difference between those snakes which became infected and those which did not was that the former were fed one newborn mouse (laboratory reared under conditions precluding contamination with *Kalicephalus* larvae) the day preceding contact with the larvae. The possible significance of this difference in technique was unnoticed until late in the study. Subsequently only a few snakes suitable for these experiments were available and these would not feed.

Discussion and Conclusions

There is a striking similarity in the development of the genital rudiment as reported by the author for *K. parvus* and *K. rectiphilus* and as described by Alicata (1) for *Hyostrongylus rubidus*. Alicata has already called attention to similar reversals of the male genital rudiment in *H. rubidus* and in *Turbatrix aceti* as described by Pai (14). The author's studies confirm the observations of Alicata and Pai in two species of another genus of the Rhabdita. It seems probable, therefore, that the reproductive systems of the Rhabdita in general develop similarly.

It is difficult to equate the statement of the Chitwoods (4) on the development of the spicules of nematodes in general with the author's observations on the spicular rudiments of *K. parvus* and *K. rectiphilus*. The author found the spicular rudiment in parasitic third-stage larvae to be a single ovate mass lying dorsal to the rectum, whereas Chitwood and Chitwood (4) comment that the spicules

> "develop in a pair of cell masses, the spicular primordia, which develop as proliferations of the dorsal wall of the cloaca, first described correctly by Seurat (1920) in *Falcaustra lambdiensis*. Schneider assumed the presence of a single primordium in nematodes with two spicules but this is incorrect."

Seurat's (16) figure of the spicular primordia of *Falcaustra* is based upon that of a fourth-stage larva. It is therefore possible that this structure begins as a single mass and divides so that it is double in the fourth stage. Indeed, the primordia of *Falcaustra* as figured by Seurat are only partially separate; they join in a single common cell posteriorly.

The author has demonstrated that experimental infection can occur by mouth. His studies also indicate that skin penetration may possibly occur. The question arises as to how entry into the host is accomplished in nature. The experimental data allow no definite conclusions, but a number of possibilities are eliminated.

It is certain that a true intermediate host is not involved in the life histories of *K. parvus*, *K. rectiphilus*, and *K. agkistrodontis*. A biological intermediate host, one in which stages in the development occur, cannot be omitted since these stages will not occur elsewhere and the definitive stage can only be derived from them. This, however, does not eliminate the possibility of a transport host being utilized in nature. In *Syngamus trachea*, which utilizes transport hosts, it is found that these hosts are not specific. Thus *Syngamus trachea* may survive in annelids, insect larvae, adult insects, and centipedes (Taylor (17), Clapham (5, 6)). Even in the Protostrongylinae, where an intermediate is obligatory, host specificity is not marked and most species can use a number of snails and slugs (Chandler, Alicata, and Chitwood (3)). The author, however, was unsuccessful in his attempts to infect earthworms, snails, insects, and mice (Schad (15)).

There remain the two modes of entry found in direct life histories, namely entrance through the mouth and entrance through the skin.

It has been found that *K. parvus* third-stage larvae are rapidly killed by drying and that the same is true for *K. rectiphilus* and *K. agkistrodontis* larvae (Schad (15)). It has also been shown that the three species develop in both aqueous and moist faeces–sand–charcoal cultures. *K. parvus* larvae reared in the latter attain the infective stage in greater numbers and survive longer than those reared in aqueous cultures. Harwood (10) has reported *Kalicephalus* larvae from various soil types in nature.

The results obtained with *K. parvus* would indicate that the conditions necessary for the survival of larvae are essentially those which are required by the ancylostomes and other species in which the larvae are rapidly killed by desiccation. The optimum conditions for these larvae are well known; a damp area exposed to little or no sunlight is the ideal environment. Under these conditions, too, kalicephalids would survive and remain viable.

The author's work shows that oral entry results in an establishment of an infection. In general, infection per os is successful when the host is a grazer. Snakes do not feed in this manner, although it may be possible that the tongue of a snake exploring the environment could introduce larvae from the substrate into the mouth. Oral infection has also evolved in the oxyurids, for example, where the life history is intimate. Development is relatively rapid and the larvae remaining in the egg are relatively resistant to adverse environmental

effects. Often the host is sedentary or regularly returns to the same den or nest. The kalicephalids studied and snakes in general do not meet these conditions.

Could larvae gain entrance to the host as a contaminant of its food? In this case one must bear in mind that snakes are strict carnivores and thus the larvae would of necessity have to be adherent to the outside of the prey. This, then, would approach ectoparasitism. However, on the basis of present evolutionary theory of nematode parasitism, such a close association should lead to the penetration of the carrier. This is especially true in such soft-skinned animals as slugs, frogs, etc. Bearing in mind the widespread occurrence of kalicephalids in snakes today, it is difficult to believe that, given sufficient time, a successful group of parasitic organisms could be intimately associated with a carrier and not have penetrated. Dougherty (7) states:

> "Most strikingly the DIAPHANOCEPHALIDAE, being specialized and at the same time restricted to snakes and lizards, suggest an antiquity of the suborder at least as great as that of the early reptiles."

This would seem too early as there are no diaphanocephalids or kalicephalids in crocodiles or turtles. However, since species of *Kalicephalus* have been reported from most families of snakes including some of the most primitive families, it is indicated that the kalicephalids arose no later than with the early snakes. The author, therefore, doubts that kalicephalids would normally enter their serpent hosts as a contaminant of prey.

Oral entrance of larvae as contaminants of water is more likely. It has been shown that larvae will develop in water and that these larvae are infective. In nature, it would seem that such conditions would arise as to permit kalicephalids to complete their life histories by being ingested with water, but the superior development of *K. parvus* larvae in a solid medium and the greater success of these larvae in the host indicates that the major route is another.

There remains the question of skin penetration. As already noted, the most likely site of successful development and survival of *Kalicephalus* larvae is one which reduces the risk of desiccation. If *Kalicephalus* larvae penetrate the skin and if the stimulus which causes the larvae to penetrate is heat, as it is in many known life histories of nematodes parasitic in mammals, then how is penetration possible into a cold-blooded snake? This problem can be resolved when one takes into account that, under certain conditions, a snake can be at a higher temperature than its environment, as, for instance, when a snake ceases basking in the sun and enters a shaded area. Benedict (2) has shown that digesting snakes maintain a skin temperature which is higher than that of the environment. He claims also that the skin of an incubating rock python registers a higher temperature than the air several inches away from the snake. The latter conclusion could not be verified in a blood python (Noble (12)).

It is apparent that a combination of circumstances can arise in which a snake enters an area contaminated by larvae and is at a higher temperature

than the immediate environment. This temperature difference would presumably be sufficient to stimulate the larvae to penetrate snakes, the probability of such an occurrence being increased by the nature of the environment necessary for larval survival.

In support of this largely speculative explanation of skin penetration is the case of the two (possibly three) snakes that were apparently infected percutaneously. As has already been noted, these snakes had been fed 12 to 24 hr. before they were placed in contact with the larvae. Then, if digesting snakes maintain a skin temperature higher than that of the environment, this could explain why larvae penetrated these snakes and not the ten subsequently exposed.

Summary

Experimental life history studies were conducted on *K. parvus*, *K. rectiphilus*, and *K. agkistrodontis*. *K. parvus* eggs cultured in tap water at room temperature hatch in 24 hr. The free-living development is completed in five days. Ensheathed third-stage larvae are found free in the stomach as late as the 10th day after infection per os. Thereafter exsheathed third-stage larvae are encysted in the mucosa of the stomach. Third-stage larvae were found so encysted in garter snakes until 130 days after infection. The fourth-stage larva was not recovered. Eggs were recovered from the faeces of an experimentally infected gopher snake after 115 days.

In tap water at room temperature, *K. rectiphilus* develops to the infective third-stage in approximately four days. In *Pituophis* spp. the parasitic third-stage was recovered from cysts in the duodenum 26 to 33 days after oral infection. At 33 days, fourth-stage larvae and subadults were recovered from the duodenal cysts of the same snake. Mature males were collected after 43 days.

The eggs of *K. agkistrodontis* are laid in the 32-celled stage. In tap water at room temperature these hatch in 24 hr. The preparasitic third-stage larvae are found in five days. The parasitic stages occur free in the lumen of the digestive tract. In 17 days, third-stage, transitional, and fourth-stage larvae are found in the small intestine. Most individuals are in a state of transition. Late fourth-stage larvae are recovered after 23 days in the host. In these, the definitive buccal capsule is developing. At the same time, immature adults are also recovered. These occur in the oesophagus. Fifty-eight days after experimental infection mature adults were recovered.

The male genital rudiment in *K. parvus* and *K. rectiphilus* was found to undergo a reversal. This structure in both sexes shows great structural and developmental similarities to the genital rudiment of *Hyostrongylus rubidus*. The spicular rudiment of *K. parvus* is described in detail. In the parasitic third stage it is a single oval structure with a posterior extension. It lies dorsal to the rectum and remains a single structure into the late parasitic third stage. This observation apparently disagrees with the findings of Chitwood and Chitwood (4) and Seurat (16), who claim that the spicular primordium of nematodes in general is a double structure.

The mode of entry into the host in nature is discussed. It is concluded that a true intermediate host does not occur and that a transport host is unlikely. Entry as a contaminant of prey is considered improbable. Ingestion of the infective stage with water is possible, but introduction on the tongue or the possibility of skin penetration is considered more likely.

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THE ROLE OF CLIMATE AND DISPERSAL IN THE INITIATION OF OUTBREAKS OF THE SPRUCE BUDWORM IN NEW BRUNSWICK

I. THE ROLE OF CLIMATE[1]

By D. O. Greenbank[2]

Abstract

New Brunswick outbreaks of the spruce budworm which began in 1912 and 1949 are considered in relation to the theory of climatic release. Studies on a natural population show that larval development is more rapid in dry and sunny weather than in humid and cloudy weather. Polar air masses bring the favorable conditions, and tropical air masses and cyclones the unfavorable. June precipitation and temperature records, analyzed in conjunction with weather maps, show that climatic changes took place in regions where the outbreaks developed. The outbreaks were preceded by dry and sunny summers during four or five consecutive years. Although direct mortality of the budworm due to adverse weather conditions has not been observed, favorable climatic conditions may have indirectly promoted population increase from the endemic to the outbreak level in the following ways. Flower production became more frequent in periods of dry years and larvae which fed on staminate flowers developed more rapidly. Larvae that developed early in the season gave rise to more fecund females than larvae that developed late. The age of current foliage consumed is also related to fecundity. The rate of development of balsam fir relative to the budworm varies from year to year. It is postulated that the average fecundity of the budworm increases in the pre-outbreak years as the result of favorable climatic conditions and greater than usual flower production. Larval mortality is greater in years with a prolonged developmental period although the increase is not statistically significant. The theory that outbreaks in New Brunswick resulted from the spread of populations from outbreak areas to the west will be considered in the second part of this two-part paper.

Introduction

The Green River Project was established in northwestern New Brunswick in 1944 for the long-term study of the spruce budworm, *Choristoneura fumiferana* (Clem.), in relation to forest management. The objects of the project have been outlined in an earlier paper (16). Climate and dispersal are among the important factors considered in these studies on the epidemiology of the insect.

Outbreaks of the spruce budworm are known to have occurred in the fir–spruce forests of northeastern North America at periodic intervals during the past 150 years. The first recorded outbreaks in this region occurred in parts of Quebec, Maine, and New Brunswick in about 1807 and 1878 (21). Recently, however, evidence has been found of earlier and previously unsuspected outbreaks and it is now thought probable that epidemics of this native insect have always formed part of the natural cycle associated with the maturing of large areas of balsam fir (6, 9).

During the almost unbroken succession of outbreaks that has plagued eastern Canada since 1909 (12), populations reached epidemic proportions in

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parts of New Brunswick in 1912 and 1949. In both instances heavy feeding damage appeared in the Province a few years after outbreaks had spread through Quebec. This west to east appearance of outbreaks on a graduated time scale is suggestive of extensive, even semicontinental, spread of infestations and from it stems a logical explanation of outbreaks of the spruce budworm in New Brunswick. The apparent spread of the most recent infestations has been recorded cartographically in the Annual Reports of the Canadian Forest Insect Survey (10). The maps suggest that the severe infestation in western Quebec in 1941 gradually spread eastward, in the direction of the prevailing wind, and reached New Brunswick in 1949. The cartography implies that infestations to the east are dependent upon high populations to the west. Whether this is a valid explanation for the origin of outbreaks in previously uninfested areas is considered in the second of these two papers.

An alternative explanation of the increases in the spruce budworm population is the theory of climatic release suggested in 1935 by Graham (11) and postulated in some detail by Wellington *et al.* (32) in 1950. According to this theory outbreaks build up in an area from the autochronous populations under favorable climate and forest conditions. The object of these papers is to show the relative importance of climatic release and dispersal to spruce budworm outbreaks in New Brunswick, and the present paper is devoted largely to climate.

History of Recent Outbreaks in New Brunswick

A standard method of dating has to be employed in comparing the events leading to the two New Brunswick outbreaks of this century. The most useful indicators of earlier outbreaks are the suppression patterns in growth rings of surviving trees along with evidence of destruction of a large proportion of the original stand (21). However, recent studies in Ontario have shown a lag of two or three years between the first severe feeding and the first reduction in radial growth in the lower stem (2). In the present study more immediate evidence of budworm feeding was used to select corresponding years in the histories of each outbreak, and by this method of dating, 1912 and 1949, the years in which defoliation was first noticeable become the 'outbreak years'.

The recorded history of the spruce budworm in this century begins with the defoliation of 1912. Any increase in population prior to this date had gone unnoticed. This outbreak, which was widespread throughout the Province, subsided in about 1920 (22). Studies were largely confined to the assessment of damage and no further mention was made of the budworm in reports until the Forest Insect Survey was established in 1937. Tree-beating collections from low-growing spruce and balsam, although not made specifically for the detection of the budworm, did not reveal its presence in the Province during the first years of the survey. Recent review by W. A. Reeks and R. S. Forbes on the number and location of subsequent collections shows that between 1939 and 1944 only 10 larvae were found in over 1000 collections made each summer

throughout the Province. Nine of these larvae were taken in collections from the northern counties, Madawaska and Victoria. Detailed population studies were begun in 1944 at Green River in the northwest. There, the first significant increase in the low level population was detected in 1947. From such information it would seem that the population of the spruce budworm in New Brunswick must have remained at an extremely low level between 1920 and 1947.

In 1947 and 1948 an infestation of the black-headed budworm, *Acleris variana* (Fern.), caused severe defoliation on balsam fir and this obscured the spruce budworm situation. However, in 1949 the loss of new foliage in several areas could be definitely attributed to spruce budworm feeding. Such defoliated areas were restricted to the northern half of the Province. The subsequent history of the present outbreak in northern New Brunswick included the development and persistence of other centers of infestation in stands of mature balsam, the sudden appearance and decline of high populations in certain stands of a non-susceptible type, the gradual spreading of the boundaries of infestation centers especially in the windward direction, and the general coalescence of infestations so that by 1953 practically all of the northern half of the Province was severely infested. The history in southern New Brunswick has been markedly different. Stands of balsam fir were considered only lightly infested as late as 1952. A small isolated outbreak did develop in Albert County in the southeast in 1953 but in the following years populations declined. Also, in 1953 there was an extension of the infestations along the coastal area into southern New Brunswick.

A detailed consideration of the course of the 1949 outbreak, especially in the so-called susceptible and non-susceptible stand types, is more pertinent to the second of these papers. However, before any consideration is given to climate to explain the pattern of the current outbreak it should be pointed out that in 1946, Balch (1) prepared a hazard map for New Brunswick which showed that the northern and particularly the northwestern regions of the Province supported far greater concentrations of mature and overmature dense softwood stands than did the southern half of the Province. The close similarity between this map and a map showing areas of severe infestation in 1951 has been discussed elsewhere (17).

Atmospheric Circulation and the Climate of New Brunswick

Atmospheric circulation processes which control the weather and climate of North America have been described by Wellington (29). The general circulation of the atmosphere tends to produce vast masses of air whose physical properties, notably temperature and humidity, are more or less uniform horizontally. Those regions of the world in which air masses regularly form are termed air mass sources. There are arctic, polar, and tropical sources which produce corresponding types of air masses and these masses may develop over maritime or continental areas. The climate of New Brunswick is predominantly controlled by the migration of those air masses that originate

TABLE I

EXAMPLES OF WEATHER CONDITIONS AT GREEN RIVER ASSOCIATED WITH THE PASSAGE OF CYCLONIC CENTERS TO THE SOUTH OR NORTH OF THE AREA

Date	Time, E.S.T.	Distance to center, miles	Pressure, mb.	Cloud cover, tenths	Cloud type	Wind	Precipitation
Cyclone 5. 1951. Developed June 26. Lat. 40°–45° north. Center passed over Great Lakes June 26, passed *south* of Green River and over Atlantic coast June 27. Lat. 40°–45° north. Fast moving.							
Green River Observations							
June 27	0730	500	1019	7	A.cu.	N.3	Nil
	1330	300	1018	5	A.cu.	NE.9	Nil
	1930	400	1020	7	Cu.	E.1	Nil
June 28	0130	400	1020	—	—	—	Nil
	0730	400	1023	10	Cu.	NE.6	Nil
	1330	450	1024	5	Cu.	NE.6	Nil
Summary:	Total amount of rain = 0 in.				No. of hours overcast = 3		
	Duration of rain = 0 hr.				Daylight hours overcast = 3		
Cyclone 1. 1952. Developed May 29. Lat. 45° N., west of Great Lakes. Center passed over Great Lakes May 31 and *north* of Green River. Slow moving east of James Bay, otherwise fast moving.							
Green River Observations							
June 1	0130	600	1029	0	0	—	Nil
	0730	500	1029	3	St.cu.	S.4	Nil
	1330	500	1028	10	St.cu +Cu.	SE.6	Drizzle 1030 hr.
	1930	500	1026	10	Nbst.	SE.2	Cont. and mod.
June 2	0130	550	1022	10	Nbst.	SE.	Cont. and mod.
	0730	600	1021	10	Nbst.	0	Cont. and mod.
	1330	650	1021	9	St.cu.	NE.4	No rain 0930–1230 Heavy shower 1230 Rain stopped 1300
	1930	750	1022	0	0	0	Nil
Summary:	Total amount of rain = 1.61 in.				Duration of rain = 23 hr.		
	Rain with heavy shower (cold front) = 0.21 in.				No. of hours overcast = 29		
					No. daylight hours overcast = 19		

over the polar continent and those that originate in the tropics over the Gulf of Mexico and Atlantic Ocean.

Polar continental air arrives over New Brunswick in the summer in a dry and clear state. If clouds form they are of the scattered convectional type that produce showery precipitation. The daily temperature range is wide because of plentiful incoming solar radiation by day and outgoing radiation by night under the clear sky. In contrast, tropical maritime air masses have high moisture values. During their northerly flow in spring and early summer they are cooled from below, and on arrival over eastern Canada are typified by high relative humidity, poor visibility, and overcast skies. The daily course of temperature is far more uniform than during invasions of polar air.

Along the borders of adjacent air masses with contrasting physical properties, areas of low pressure, cyclones, which contain complex frontal systems frequently develop. Frontal weather is markedly different from the air mass weather found on either side. The circulation of a cyclone causes the warmer air mass to override the colder air mass in one sector of the system, the warm front, while colder air pushes forward behind the warm air mass in another sector, the cold front. Such a circulation system which causes warm, moist air to rise and creates frontal surfaces is a natural 'rain maker'.

Strong cyclonic activity over New Brunswick in the summer is always associated with cool, wet weather. Markedly different weather results when cyclones bypass the Province depending upon whether their courses are to the north or south, as observations taken from the files of the Green River weather station clearly show. When a cyclone approaches from the interior and eventually passes to the south, the circulation system around it causes polar air, either maritime or continental, to flow over the area; the frontal surfaces do not pass over the area.

When a cyclone center passes to the north of the Province the wind pattern causes humid tropical air to flow over the area. The frontal surfaces, if well developed, pass over the Province and cause continuous precipitation. The relative effects of cyclone centers passing to the south and north of the area are shown in Table I.

It is readily understandable that climatic variation in New Brunswick results from changes in the relative frequencies of invasions by tropical and polar air masses and from changes in the intensity of cyclonic activity. Summers will be dry and sunny if polar air penetrates more frequently, whereas summers will be cloudy and wet when tropical air predominates or when cyclonic activity over the area is intense.

Weather and the Spruce Budworm

Recent laboratory and field investigations in Ontario have shown the effects of light, temperature, and moisture upon the behavior and activity of the spruce budworm (24, 25, 26, 27, 33). Their findings may be summarized very briefly by saying that under moist and wet conditions larvae do not feed readily but remain relatively inactive, whereas under dry and sunny conditions feeding becomes more continuous. Davis (8) in New Brunswick used coprometers to measure the feeding activity of fifth- and sixth-instar larvae. His records show that with low temperature and high relative humidity there was little or no feeding, whereas with higher temperatures and low humidity there was considerable feeding.

These conclusions have been tested at Green River by studying the effect of weather, rather than individual physical factors of the environment, on a natural field population. The progress of larval development is followed in an area by taking samples about every third day and recording the percentage of the population in each instar. The progress through each instar becomes evident when such percentages are plotted over dates (Fig. 1) and the duration of each instar can be read along with measurements of each larval stadium. For example, the duration of the fourth instar is the time interval between the first molting into the fourth instar and last molting into the fifth instar. One measurement of the fourth stadium is the time interval between the first larva entering the fourth instar and the first larva entering the fifth instar; another measurement of the fourth stadium is the time it takes the last larva that enters the fourth instar to proceed to the fifth instar. Thus for each instar in any year two measurements of the stadium are taken. For this

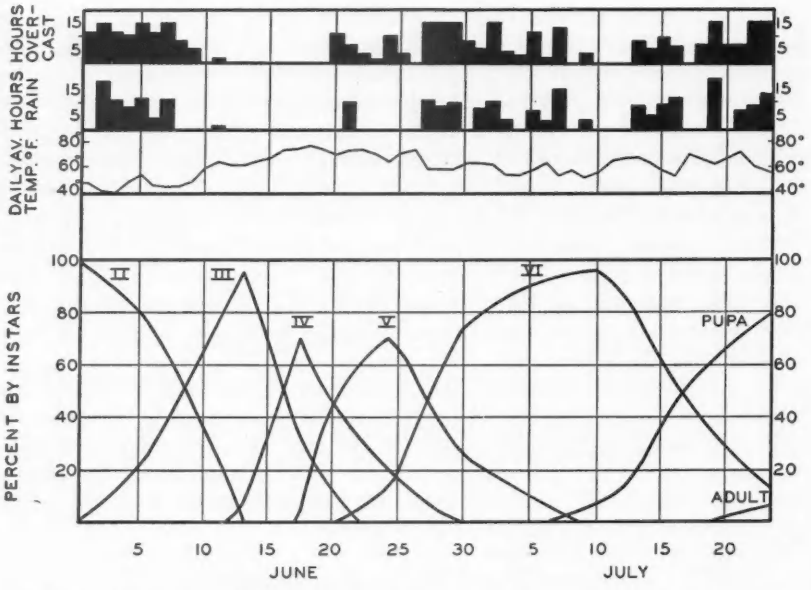

FIG. 1. Percentage distribution of the larval population according to instars and weather conditions at Green River in 1954.

study of larval development it was believed that more accurate measurements of the larval stadia could be obtained by considering the time it took 20% of the population to proceed from one instar to the next. By this displacement in the basal axis of the graph from the 0% level to the 20% level the tail ends of each instar developmental curve are ignored. The shallow nature of these tail ends makes it difficult to determine where they intersect the horizontal axis, and this is due in part to the fact that a few larvae sometimes remain alive for a considerable time and yet never proceed to the next instar. Such records of larval development have been maintained for the past six years, 1950–1955, along with continuous weather records. The 12 measurements of each larval stadium, two measurements taken in each of six years, may be related to the weather prevailing during the individual developmental periods.

Air mass weather, either polar continental or tropical maritime, and frontal weather may be readily distinguished in records of air temperature, rainfall, and cloud cover. The actual attributes of weather measured were:

Average day-time air temperature.—This is the average of two-hourly readings taken between 0600 hr. and 2000 hr.

Duration of rainfall.—The total number of hours during which rain fell.

Hours of overcast sky.—The total number of daylight hours, between 0600 hr. and 2000 hr., during which skies were completely overcast.

The choice of attributes resulted partially from the findings of previous investigators, but essentially from the fact that when considered together

they indicate the type of weather prevailing. Average correlations among these attributes are all highly significant. Prolonged rainfall is associated with prolonged periods of overcast sky and, under such conditions, daytime temperatures in the summer do not reach those heights common to clear sky conditions. The interrelationship can be seen in Fig. 1. Previous investigators had shown that most of the larval feeding takes place during the daylight hours. Rainfall during the night, however, may be important to larval feeding because feeding sites often remain saturated for a considerable time after prolonged rainfall. Hours of rainfall can also be measured throughout the day and night with a recording rain gauge, whereas direct observations on sky conditions are more or less restricted to the daytime. For these reasons cloud cover was observed and recorded only during daylight, whereas rainfall amount and duration were recorded throughout the 24-hr. period.

The correlations between larval stadia and prevailing weather conditions are shown in Table II. Rates of development increased significantly in the third, fourth, and sixth instars with increases in the average daytime temperature. Rates of development were retarded significantly in the third, fifth, and sixth instars with prolonged hours of rain and overcast sky. The correlations between hours of rain and larval stadia are actually cumulative correlations in that there is an interdependence of the stadia (y) and hours of rain (x), hours of rain being partially dependent on the length of time being considered. It is the same with hours of overcast sky. This interdependence of variables could be eliminated by expressing the duration of rainfall as the average hours of rain per developmental day. However, erroneous conclusions as to the effect of rainfall on development may be drawn when this average is used—as an example helps to demonstrate.

In 1951 the fourth stadium was six days. Rain fell for 14 consecutive hours with the passage of a warm front, and brief showers during the remainder of the period accounted for another eight hours of rainfall. The total duration of rainfall was 24 hr. and the average was four hours per day. In 1952 the fourth stadium was 10 days. After five days without rain a cyclone became

TABLE II

THE AVERAGE STADIA OF SPRUCE BUDWORM LARVAE AND THE CORRELATIONS BETWEEN STADIA AND PHYSICAL ATTRIBUTES OF WEATHER

Instar	Av. stadium in days	Correlation coefficients		
		Stadium : Temperature	Stadium : Hr. rain	Stadium : Hr. overcast
III	10.00	−.770**	.718**	.746**
IV	7.50	−.648*	.379	.583
V	7.25	−.252	.710*	.709**
VI	16.70	−.789**	.939**	.900**

*Significant within the .05 level.
**Significant within the .01 level.

TABLE III

THE LARVAL PERIODS, THIRD INSTAR TO SIXTH INSTAR, AND THE DAYLIGHT HOURS OF OVERCAST SKY, HOURS OF RAINFALL, AND AVERAGE DAYTIME AIR TEMPERATURES FOR THE YEARS 1950–1955 AT GREEN RIVER

Year	Larval period	No. days	Daylight hr. overcast	Hr. rain	Av. daytime air temperature, ° F.
1950	May 26–July 12	48	236	235	61.2
1951	May 26–July 13	49	245	200	60.9
1952	June 4–July 9	36	165	131	64.4
1953	June 1–July 11	41	173	96	62.9
1954	June 5–July 22	48	290	203	62.3
1955	May 21–July 2	43	204	197	62.5

stationary over the area and rain fell for 40 hr. during the next three days. The average was again four hours per day, whereas the actual duration was 40 hr. Therefore, the effect of rainfall on development is better shown by total duration, in spite of its limitations, than by the average daily duration. For a similar reason the use of the total hours of overcast sky in a developmental period can be defended.

Larval periods can also be compared on a seasonal basis. Table III shows the duration of the larval stage, from the beginning of the third instar to the end of the sixth instar, and weather conditions for each year. Larval periods were briefer in summers that were clear and dry and had high daytime temperatures. The combination of these correlations shows that under clear and dry conditions the budworm develops more rapidly than under wet and overcast conditions. These were precisely the same conclusions drawn by Wellington. Clear and dry weather is common to polar air, whereas wet and overcast weather is common to both tropical maritime air and fronts. It is of little importance which of the physical attributes is most effective in determining the rate of larval development. All of the attributes are interrelated, respond together, and go to make up weather.

The Theory of Climatic Release

Wellington *et al.* (32), after distinguishing between those weather types favorable and unfavorable to the budworm, related past outbreaks of the spruce budworm in central and eastern Canada to climatic changes. It was shown that outbreaks were preceded by reductions, during three or four consecutive years, in the annual numbers of cyclonic centers passing through the affected areas and by reductions in June precipitation. By later more refined weather analyses, Wellington (28, 29) has been able to show definite short-term latitudinal shifts in the movements of pressure centers over North America. Pressure centers are closely associated with known air-mass source regions and may be differentiated into groups depending upon where they originate. When the tracks for each group of centers are traced and studied

independently, shifts in the principal courses from one period to the next frequently show up. A southward displacement of the tracks of those centers originating in the central States, 'Colorado lows', is associated with a corresponding southward displacement of the tracks of pressure centers originating over the polar region. Humid tropical air masses will be mostly barred from the Great Lakes region when such a southward shift of the circulation pattern occurs in the central part of the continent. The majority of air masses that pass over this region will then be of polar origin. For polar air to penetrate more frequently than tropical air into New Brunswick, which lies in the major exit channel of North American weather, these southward shifts must be further associated with a shift to the south and east of the tracks of pressure centers originating within the Gulf and Atlantic source of tropical maritime air. These pressure centers generally hug the Atlantic seaboard but are subject to periodic shifts either inland or seaward.

The jet stream is one important factor that determines whether an area such as New Brunswick shall come predominantly under the influence of the favorable polar air or unfavorable tropical air. This recently discovered meandering belt of high-altitude and high-velocity wind has a guiding influence on the pressure systems that develop at the earth's surface. Displacements in the mean course of the jet stream whereby it is farther south or north than normal in successive summers may result in a more or less orderly variation in climate over short periods, that is from dry, clear summers through a cloudier, moister period to a number of wet summers.

These findings of Wellington show the relation of climatic changes to the initiation of past outbreaks of the spruce budworm in the Boreal Forest of Ontario and Quebec. "For the spruce budworm to develop large populations it is first necessary for the forest to reach the stage where large stands of overmature host trees exist. However, in areas where these stands occur no initial increase seems to occur until seasonal climatic control of the indigenous, small population is relaxed by a change from abnormally or normally wet, cloudy weather to relatively dry, clear weather. If such weather recurs several years in succession the enormous potential for increase that the species possesses is realized and the population grows so rapidly that no combination of adverse physical and biotic factors can bring it under control immediately" (30).

The theory of climatic release explains the time and place of budworm outbreaks and its worth will be measured by its ability to predict outbreaks. It is not a theory to explain the regulation of an insect population at all levels of abundance, comparable to those theories of natural control reviewed by Solomon (20). The purpose of the theory is not to postulate regulation of population by climate. Studies on population dynamics in forest entomolgy during endemic periods are rare (19) although it is apparent that fluctuations in numbers without loss of balance are common and outbreaks the exception. Within the endemic period increases in population from one year to the next can result from physical conditions becoming favorable to the insect. Readjustment of the population after this increase may come through density-

related processes although these may not be entirely effective until physical conditions become unfavorable again. However, years with unfavorable weather conditions cannot always be expected to follow years with favorable conditions and eventually the favorable weather conditions recur several years in succession. During such a period, as the theory of climatic release postulates, the endemic population may be released from the controlling influences of both physical and biotic factors.

The relation of the beginning of insect outbreaks and favorable climate has been suggested several times in the past, particularly by German entomologists (19, 23). However, thorough tests of this relationship for a species have been lacking until Wellington began studies on the spruce budworm.

Climatic Changes in New Brunswick

There are several ways in which the incidence of frontal passages and air-mass types passing over New Brunswick can be investigated. Daily records of daytime air temperatures, hours of overcast sky, and hours of rainfall were utilized in determining the effects of the different weather types on larval development. Unfortunately long-term records of such detailed observations are not available, but continuous records of precipitation and air temperature for numerous stations are published in the Monthly Record of the Meteorological Division, Department of Transport, Canada. Also, maps showing the path of cyclonic centers, published in the Monthly Weather Review of the United States Weather Bureau, can be utilized in the reconstruction of past weather patterns. Because in New Brunswick the greater part of the larval stage is passed in June, only weather records for that month have been analyzed in this study.

Precipitation totals may be taken as a rough indicator of available moisture. The earliest records of June precipitation in New Brunswick date back to 1875. Only six weather stations were then in operation compared with some 50 today. June precipitation has been studied to see if any change in pattern can be related to the outbreak years of 1912 and 1949. Since precipitation as registered at several stations is more representative of provincial conditions than at just one station, all stations with 30 years of continuous records have been combined. The span of 30 years for the establishment of means was set by the International Climatological Commission. For the 1912 outbreak, precipitation for each June during the years 1887–1916 has been compared with the June mean for that 30-year period. Similarly for the 1949 outbreak June precipitation values during the period 1924–1953 have been compared with the June mean of that period.

Absolute values of precipitation cannot be used to show trends on a provincial basis because amounts of precipitation consistently vary from station to station (31). For each station the annual percentage deviations from normal were first determined. Station deviations were then combined to show regional trends. These percentage deviations are plotted with their four-year running averages in Fig. 2.

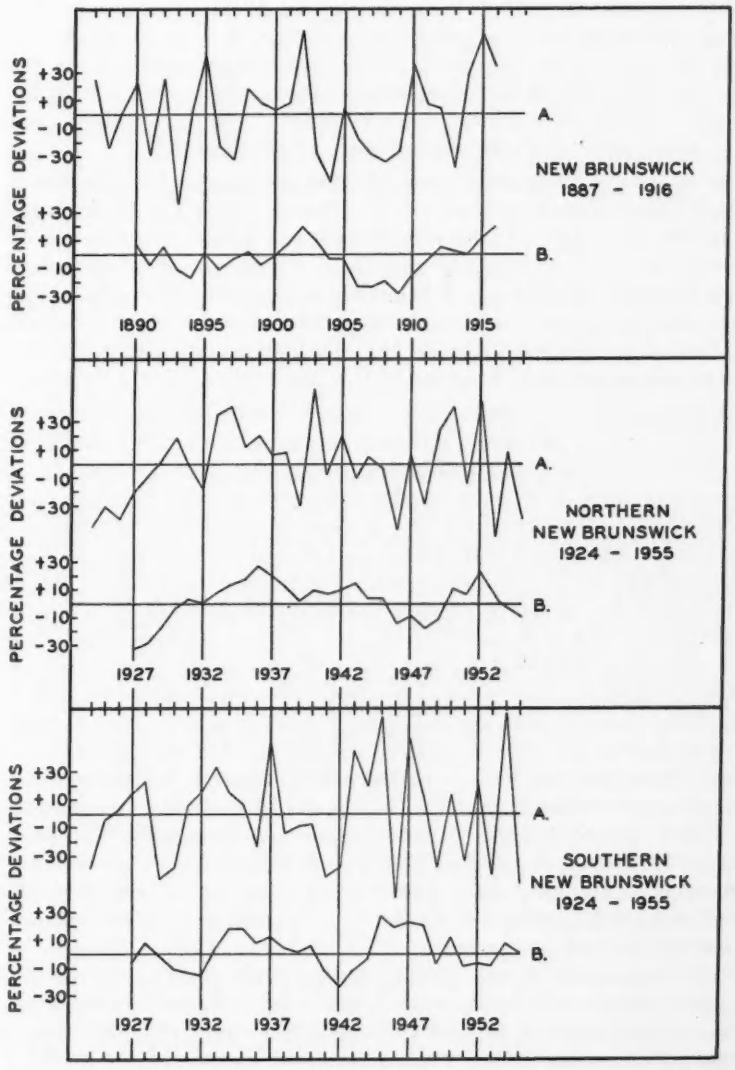

FIG. 2. June precipitation records for New Brunswick during 1887 to 1916, for northern New Brunswick 1924 to 1955, and for southern New Brunswick during 1924 to 1955.

Graphs A. The percentage deviations from normal June rainfall plotted over years.
Graphs B. Four-year running averages of percentage deviations from normal June rainfall plotted over the fourth year.

The records of four stations, two in southern New Brunswick and two in northern New Brunswick, were studied for the 1912 outbreak. All stations showed fluctuations from positive to negative deviations up until 1902. There was a period of decreasing or subnormal June rainfall from 1903 to 1909. On the basis of four-year periods, June deficiencies occurred for the four years ending 1904 to four years ending 1911. No such lengthy sequence of dry Junes occurred at any other time in the 30-year period.

The records of 12 stations were analyzed for the period 1924–1953. No drought period immediately prior to the 1949 outbreak became evident until the records had been divided into groups, one group consisting of the six northern stations and the other group of the six southern stations. The period 1943 to 1948 was one of decreasing or subnormal June rainfall in the north while excesses were common in the south. An earlier dry period showed up in the south between 1938 and 1942 when the north of the Province was experiencing above-normal precipitation.

Further resort was made to meteorological records to make sure that these dry periods represented shifts in the circulation pattern. It is to be expected that under clear sky conditions, so typical of dry polar air masses, diurnal temperature ranges will be greater than under the more overcast conditions of humid tropical air. After extraction of mean daily ranges of June temperature from the monthly record, chi-square tests showed the association of increases in temperature range with decreases in precipitation to be highly significant. Thus daily ranges of temperature can be used along with precipitation values as an indicator of past weather. In the years immediately preceding the 1949 outbreak greater than normal ranges of June temperature were recorded by the northern stations in the years 1944, 1946, 1947, and 1948, while in the south only June of 1946 showed greater than normal. Temperature and precipitation trends when considered together in this manner indicate that northern New Brunswick came predominantly under the influence of polar air during this pre-outbreak period while tropical air masses and frontal passages penetrated more frequently into southern New Brunswick. The drought between 1938–1942, as reflected in the precipitation pattern of southern New Brunswick, is probably not a good indication of the type of air mass that prevailed over the region. Only 1939 and 1942 showed slightly greater than normal ranges of daily temperature. Precipitation and temperature records do show a pronounced dry period, during which polar air predominated, which was not followed by an outbreak of the spruce budworm. This occurred between 1922–1928, but most of the susceptible stands were killed just prior to this period by the 1912–1920 outbreak.

Tracks of pressure systems over North America for the years prior to the 1949 outbreak have been examined and found to support, to some extent, the conclusions drawn from precipitation and temperature data. Wellington's illustrations (29) of these tracks show that in the period 1943–1945 the production of centers of all types was low. Centers of polar origin did not penetrate far south and so would not influence southern New Brunswick as

much as northern New Brunswick. In the period 1946–1948 centers of polar origin predominated over New Brunswick. However, this southward shift of the polar group was not accompanied by an eastward shift of tracks of tropical centers of Atlantic and Gulf origin. Consequently, southern New Brunswick would not necessarily experience as great a climatic change as northern New Brunswick.

These findings in New Brunswick strongly support those made by Wellington on the time and place of spruce budworm outbreaks. The outbreak of 1912 was widespread throughout the Province and followed several years of dry, sunny June weather. The 1949 outbreak was confined to the north where dry and sunny weather had been experienced for several years. Currently no serious outbreak has developed in the south nor has this region experienced a favorable climatic change. However, a susceptible forest is a prerequisite of budworm outbreaks. Continuous areas of mature balsam exist in northern New Brunswick, whereas in general stands in the south are more broken, are of a younger age-class, and contain relatively higher percentages of other species.

The Mechanism of Climatic Release

The relation of favorable climate and forest insect outbreaks has been demonstrated both for the budworm and other insects. The actual mechanism by which climate may permit a release of population has never been satisfactorily shown. Evidence is presented here to demonstrate how climate may influence the population density of the spruce budworm. With the basic assumption that population density is the result of fecundity on the one hand and mortality on the other, the influence of climate will be examined more fully.

Climate and Fecundity

The average fecundity of several lepidopterous species changes appreciably from generation to generation even when there is an abundant supply of food (19). Significant changes in the average fecundity of generations of the spruce budworm have been observed only when food supply is limited. However, few measurements have been made when there was an abundant supply. Nevertheless studies have shown that the fecundity of the spruce budworm is at least partially dependent upon feeding conditions, and these are known to change from year to year. In some years larvae complete their development early in the season and feed on shoots before growth is complete, while in other years larval development is delayed in relation to shoot growth. The age of foliage at the time of consumption has a relationship to fecundity. Variation in feeding conditions result from seasonal differences in the phenologies of the host and the insect.

Changes in Fecundity

The importance of food quality to the fecundity of the spruce budworm has been demonstrated by several investigators (5, 14). More eggs are laid by budworm that have developed on current foliage than budworm which,

in their later larval stages, have been forced to feed on foliage of previous years. Experiments were conducted at Green River to determine whether the food quality of current foliage also deteriorates as the season progresses.

Living female pupae were collected throughout the pupal period from three plots in 1955. These were placed individually in sputum bottles and stored in the insectary. A total of 465 moths emerged. On emergence each female was paired with a freshly-emerged and unmated male in an oviposition cage. This was simply a lantern globe containing a 6-in. branch tip of balsam fir secured in an upright position. The cut end of the branch was inserted in a small vial of water. Only 218 or 47% of the experiments were considered successful. Injuries to females, failure to mate, and the death of females retaining practically a full complement of fertile eggs accounted for the many unsuccessful experiments. To derive the most information from the collected data, the relation of pupal size to fecundity was established. The depth of the second segment of the empty pupal case was taken as the index of size (14). A total of 197 pairs of values of the two variates was available. Analysis of the number of fertile eggs laid and the pupal size showed strong positive correlation on each of the three plots (Table IV). The regression lines are plotted in Fig. 3. After tests of homogeneity of data, the plot regressions were combined to give an average regression, the best available estimate of the true population regression. This average regression has a coefficient of 126 eggs per millimeter of pupal size and a standard deviation from regression of 46 eggs. The same regression coefficient was found by Miller (14) but in his experiments more eggs were laid per female. This is believed due to differences in experimental design. In Miller's design the upright balsam twig extended to the base of the oviposition cage making it relatively easier for the female moth to climb on to the foliage. In the present design the foliage was raised three inches above the base of the cage by the water container. Partially spent females had difficulty in reaching the foliage and probably did not lay their full complement of eggs.

Several collections of living pupae were made at each plot throughout the pupal period. The individuals that had developed most rapidly were taken in the first pupal collections; those that had developed at a slower rate and

TABLE IV

REGRESSION AND CORRELATION DATA OF PUPAL SIZE ON FECUNDITY IN THREE PLOTS IN 1955

Plot	I3	K3	G6	All plots
No. of experiments	120	32	45	197
Mean pupal size	3.297	3.003	3.296	3.249
Mean fecundity	185.0	138.8	182.7	177.0
Correlation coefficient	0.442**	0.735**	0.560**	0.530**
Regression coefficient	109.79	149.73	135.78	125.78
Standard deviation from regression	46	37	51	46

***Significant within the .01 level.*

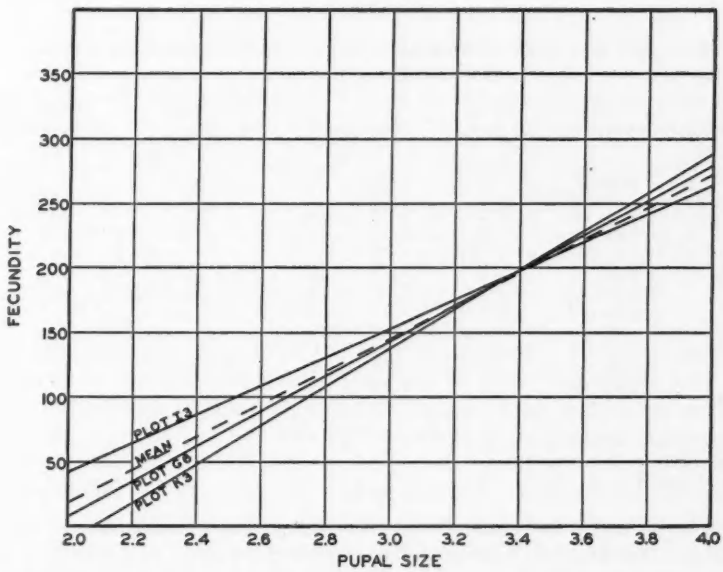

FIG. 3. Regressions of fecundity and pupal size in three plots and the average of these regressions.

pupated later in the season were taken in the last collections. Comparisons revealed that the average pupal size of the first collection was greatest and the average size of each subsequent collection was smaller (Table V). By July 8 some adults had already emerged in the field and so two pupal collections were made at Plot 13. The first was the regular collection of living female pupae and the second a collection of the empty female pupal cases of the current year. The difference in average pupal case size of the two collections was highly significant. After conversion of pupal size to fecundity, using Fig. 3, it was estimated that the early emerging adults laid an average of 21 eggs more than the females that emerged after July 8.

Pupal state	No.	Mean size	Difference in pupal size	Difference in fecundity
Emerged	55	3.42±0.20*		
Non-emerged	80	3.26±0.19	0.17	21

* *The measure of variation used in tables is the standard deviation.*

Defoliation of the current foliage on the three plots after completion of larval feeding was below 80%. The gradual reduction in pupal size and fecundity of the generation need not therefore be attributed to the effect of feeding on foliage of previous years by the slow-developing larvae.

TABLE V

MEAN SIZE OF FEMALE PUPAE COLLECTED IN THREE PLOTS AS LIVING PUPAE

Plot	Date of collection	No. of pupae	Mean size	Difference in pupal size	Difference in fecundity[1]
I3	June 27	12	3.48 ± .20		
	July 3	123	3.33 ± .19	0.15**	19
	July 8	80	3.26 ± .19	0.07**	9
	July 12	30	3.13 ± .17	0.13**	16
K3	July 5	18	3.18 ± .14		
	July 17	92	2.99 ± .21	0.19**	23
	July 22	19	2.72 ± .24	0.27**	35
G6	June 28	27	3.42 ± .15		
	July 5	45	3.32 ± .25	0.10	14
	July 12	19	3.12 ± .21	0.17*	21

Significant within the .05 level.
**Significant within the .01 level.*
[1]*Read from Fig. 5.*

As first-formed pupae were found to be larger than pupae formed later in the season, an attempt was made to express differences in pupal size on a temporal basis. Each female pupa collected in the field was isolated in the insectary and the date of adult emergence recorded. If it is assumed that the daily order of adult emergence in the insectary is a reflection of the daily order of pupation in the field, then the regression of pupal size on the order of pupation can be determined (Table VI). For each plot the correlation between pupal size and order of pupation was found highly significant, and under the experimental design used for establishing the relation of pupal size to fecundity it may be stated that the average fecundity of the spruce budworm was from four to six eggs lower for each day that the developmental period was extended.

Results from these studies have indicated a definite relationship between fecundity and development, and this had been suspected by earlier workers (8, 14). It was found that the first-formed females in an area were more fecund than females formed later, even though all individuals developed on

TABLE VI

REGRESSION AND CORRELATION DATA OF PUPAL SIZE ON ORDER OF PUPATION IN THREE PLOTS

Plot	I3	K3	G6	All plots
No. of pupae collected	300	129	91	520
Regression coefficient	−0.038	−0.046	−0.039	−0.041
5% Fiducial limits	−0.048	−0.058	−0.050	−0.047
	−0.028	−0.034	−0.028	−0.035
Correlation coefficient	0.401**	0.542**	0.596**	0.487**

**Significant within the .01 level.*

TABLE VII

COMPARISON OF FECUNDITIES OF SPRUCE BUDWORM REARED ON EARLY AND LATE CURRENT FOLIAGE

Diet	No. of budworm	Degrees of freedom	Mean fecundity	Sum of squares
Early foliage	39	38	168.9	107,308
Late foliage	28	27	102.9	51,612
		Sum = 65	Diff. $\bar{x}$ = 66.0	Sum = 158,920

NOTE: $S\bar{x} = 12.2$; $t = 5.4$; $P < 0.01$.

current foliage. Further evidence that the age of current foliage is an important factor in causing the reduction of fecundity comes from the following experiment. Second- and third-instar larvae were collected in the field in late May and early June. These larvae were then reared on current foliage through to the adult stage in the insectary. Of 39 issuing females the mean fecundity was 169 eggs. A second group was kept in cold storage and held back in hibernacula until the first group had pupated. The larvae of the second group were then reared on current foliage to the adult stage. Of 28 issuing females the mean fecundity was 103 eggs. The rearing technique was identical for the two groups of larvae. The foliage was collected from the field in both cases and renewed every three days. However, the current foliage had completed its seasonal growth by the time it was fed to the second group of larvae and was therefore relatively older than the foliage fed to the first group. The mean difference of 66 eggs per female between the two populations was highly significant (Table VII).

Food consumption tests were conducted using larvae from the early and late groups above. Freshly-molted sixth-instar larvae were placed individually in vials with a balsam fir shoot. The shoots were weighed just prior to the start of each experiment and the cut ends were inserted in a reservoir of water to prevent drying of foliage. On pupation the shoots were weighed again. Three controls were used for each group to determine the increase in weight due to absorption of water. From the 23 tests it was shown that a greater weight of foliage was consumed by larvae given a diet of the relatively younger current foliage. Also, these larvae formed larger pupae than larvae fed the relatively older current foliage.

Larval Development

It has already been shown that during dry and sunny weather larval feeding is more continuous and development more rapid than in humid and overcast weather. Furthermore, several investigators have shown the importance of staminate flowers to the development of larvae in the early instars (3, 4, 13). Blais (4) found that larvae which fed on pollen had an advantage of about three days in development over larvae feeding exclusively on foliage. With few exceptions balsam fir on the Green River Watershed has borne heavy

crops of flowers every second year. This same periodicity occurs at widely-scattered points throughout the northeast in New York, Vermont, Maine, and New Brunswick (7). Nevertheless recent studies on the frequency and abundance of flower production have shown that the regular flowering year/non-flowering year rhythm may be broken in periods of dry summers when years of moderate flower production follow heavy flowering years. Such was the case between 1944 and 1948 when flowers were produced in four of the five years. Morris (15) earlier had traced the flowering history back as far as 1920 and found that the periodicity was also broken in 1927 when flowers were produced in three consecutive years. New Brunswick at the time was experiencing its worst summer drought on record. Thus it may be assumed that in the years immediately prior to the 1949 outbreak conditions were ideal for rapid larval development. This resulted from a direct climatic effect as well as increased flower production in response to climate.

Shoot Development

It has been shown that the age of foliage of the current year at the time of consumption is important to fecundity. The manner and rate of the deterioration of food quality as the season progresses is not known. It is possible that as shoots elongate and approach completion in growth they simply become less palatable to the budworm because of the hardening of the needles. Shoot growth in such a case would be an index of age and food quality. Curves of shoot growth are constructed annually at Green River in the course of phenological studies conducted by Morris and Bennett (unpublished).

Shoot development may be followed by measuring the length of shoots on different dates throughout the growing season. When the initial length, that is the length before the buds begin to elongate, and the length of the shoot after growth is complete are known, then the length of any intermediate date can be expressed as a percentage of the total shoot growth for that season. The curve of cumulative shoot growth is sigmoid with that portion between 25 and 75% being relatively straight. The growth curves between these limits are plotted as broken lines in Fig. 4 for the years 1950 to 1955. Minor weather changes are not reflected in these curves but the steepness of slope denotes the favorability of seasonal conditions for growth.

Relative Phenologies

The phenologies of balsam fir and the spruce budworm can be compared for an area by constructing the curve of larval development alongside the curve of shoot development. Larval development is shown in Fig. 4 where the dates on which the population had reached the end of each instar are plotted. These data were taken from development curves similar to that illustrated for 1954 in Fig. 1. The phenological curves show that in 1950 and 1955 larval development was generally more advanced in relation to shoot development at Green River than it was in other years. In those years the larvae fed on foliage that was relatively young. In 1952, weather conditions became unusually favorable for rapid larval development in the first week of

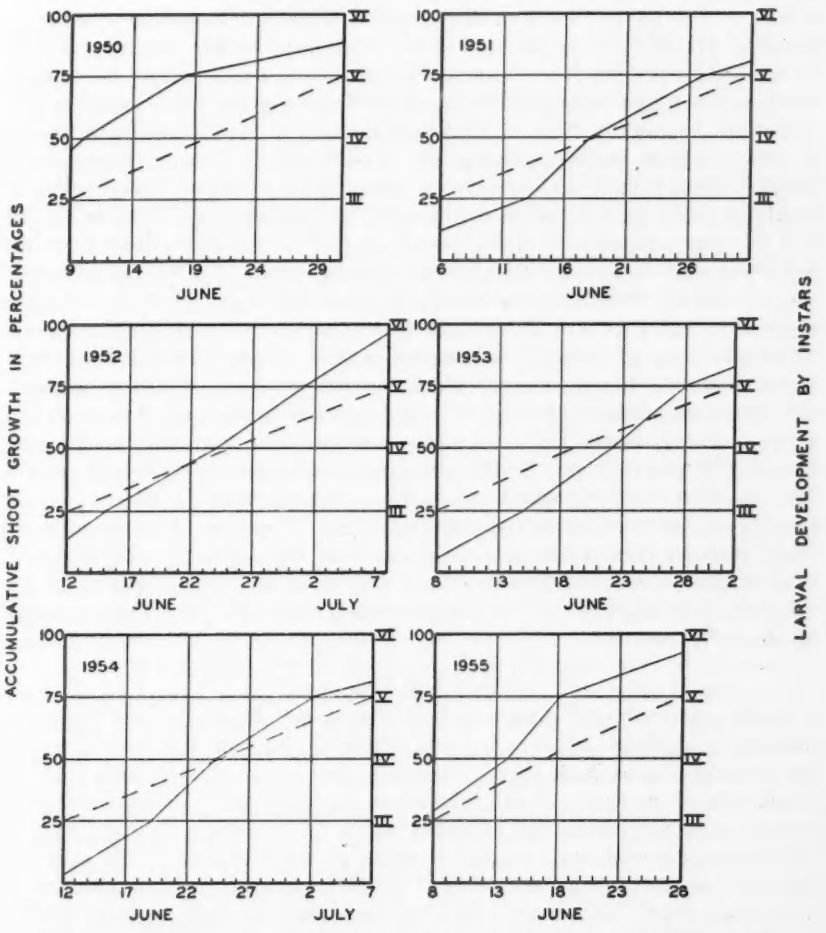

FIG. 4. The relative phenologies of balsam fir and the spruce budworm at Green River for the years 1950–1955. Curves of the accumulation of shoot growth from 25% to 75% are plotted as broken lines. Curves of larval development that show dates on which the budworm population had completed development in the third, fourth, fifth, and sixth instars are plotted as continuous lines.

July and by the time 75% shoot growth was complete practically all the population had proceeded to the pupal stage. It is evident that current foliage is not in the same state of development each year at the time of feeding.

In the spring the initiation of larval growth is not always closely synchronized with the initiation of shoot growth. Emergence from hibernacula has preceded the beginning of shoot growth by as much as 16 days at Green River. Seasonal variation in the phenologies of the spruce budworm and its host may result simply from such differences in these initial phenological

events. The relative rates of development of the larvae and its host may subsequently decrease or increase these differences. When emergence from hibernacula precedes shoot growth, second-instar larvae resort to mining needles, vegetative buds, and/or staminate flowers in the intervening period. In flowering years the effect of a prolonged mining period might prove beneficial to the population for young larvae thrive on a diet of staminate flowers and develop more rapidly than larvae in other feeding sites. Consequently, in flowering years, if emergence is early and the start of shoot growth late, larvae will be more advanced in development relative to the shoots than in years when the two events are more closely synchronized. In the pre-outbreak years, 1945 to 1948, which were mostly flowering years, phenological data show that shoot growth began late in comparison with succeeding years. Although dates of budworm emergence in these years are not known, it is highly probable that the event took place relatively early. With clear polar air predominating over the region in these pre-outbreak years, foliage at the crown canopy would be warmed by radiant heat and emergence from hibernacula encouraged. At the same time, air temperatures would remain low, especially in dense stands where snow coverage persists under polar air conditions, and shoot growth would be retarded. Therefore, it seems probable that, through climate, larval development in the pre-outbreak years was always more advanced relative to shoot development. Such a relation of the two phenologies favors high fecundity provided staminate flowers are available to the early instars.

Climate and Mortality

It is estimated that populations of the spruce budworm can rise from endemic to epidemic levels in a period of four or five years. It can be shown by the following example that increased fecundity cannot bring about such a rapid rate of change in population unless the increase is accompanied by a reduction in the percentage mortality occurring in each generation. If, in the endemic period, the average fecundity of the budworm is 150 eggs per female, then a generation mortality of 98.7% is needed to hold the population at a steady level. It is conceivable that the average fecundity might be raised as high as 200 eggs per female in the pre-outbreak years when climatic conditions become favorable. After five years, assuming percentage mortality remained unchanged, this would raise the population level only about fourfold. However, if through an increase in fecundity, the mortality factors became less effective and removed 96% instead of 98.7% of each generation, then after five years populations would have increased over a thousandfold. It is apparent that for an outbreak to develop within a few years a reduction in percentage mortality in the pre-outbreak years is essential. Thus, if we postulate that climatic release operates through its effect on fecundity, we must also postulate that an increased fecundity, when sustained for several consecutive years, has an influence on the efficacy of certain mortality factors.

Direct mortality of the budworm resulting from extremes in physical factors is seldom observed. In parts of New Brunswick in 1953, where

severe spring frost had killed the expanding buds of balsam fir, the mining larvae appeared unharmed (18). The overwintering mortality of larvae in hibernacula seems to change little from one year to the next regardless of variation in winter conditions. Moths appear unharmed after being transported on convectional storms and fly strongly in the heavy rains associated with such weather. Furthermore, once a population is released from its endemic level, adverse physical factors do not seem to bring it under control. Both the 1912 and 1949 outbreaks finally reached their peaks during periods of wet years.

There are undoubtedly many indirect ways in which climate is related to mortality. For example, if the larval period is prolonged through adverse weather conditions then the host population is subject to a longer period of attack by natural enemies. Larval mortality under such conditions would increase although it may be argued that the weather conditions that retard the development of the host would also retard the activity of insect predators and parasites. However, the effectiveness of other biotic factors, such as birds and disease, would no doubt be greater over the extended period. Life tables are constructed at Green River for successive generations of the budworm in various stands and show the mortality occurring in different stages of the life cycle (16). Reductions in population from the third instar to the pupal stage have been determined for each plot and expressed as percentages. When the same plots are studied in successive years then the mean difference in mortality between the two years can be tested for significance using a standard error calculated from the interaction mean square. For present purposes, this analysis must be restricted, of course, to plots which had such a low population that there was no competition for food. Plots, in such an analysis, are treated as replicates. Comparisons of the mean percentage reductions in population occurring in successive years are shown in Table VIII. It is readily seen that the year which had the shorter larval developmental period also had the smaller mean percentage reduction in population. However, the mean differences are not statistically significant and definite conclusions may not be drawn until further life tables are constructed for low or moderate populations.

TABLE VIII

COMPARISONS OF MEAN PERCENTAGE REDUCTIONS IN POPULATION FROM THE THIRD INSTAR TO THE PUPAL STAGE AND COMPARISONS OF THE LARVAL DEVELOPMENTAL PERIODS IN SUCCESSIVE YEARS AND IN THE SAME PLOTS

Year	No. plots	Mean per cent reduction	No. of developmental days	Year	Mean per cent reduction	No. of developmental days
1950	4	72.9	48	1951	82.4	49
1951	5	83.6	49	1952	76.5	36
1952	5	76.5	36	1953	80.7	41
1953	5	81.6	41	1954	90.5	49
1954	5	89.6	49	1955	74.4	42

Discussion

There is evidence of outbreaks of the spruce budworm in at least some parts of New Brunswick beginning about 1770, 1806, 1878, 1912, and 1949. Radial growth patterns suggest that the outbreaks lasted about 10 years. For the greater part of its history the budworm has remained at the endemic population level and little is known of the insect in this important period. Survey collections showed that the budworm, rather than disappearing altogether from the province, was at least present in small numbers in parts of New Brunswick between the two outbreaks of this century.

The analysis of weather data for the years immediately preceding the 1912 and 1949 outbreaks shows that climatic conditions became favorable for the budworm in those areas where the outbreaks began. This association of regional climatic changes and the development of outbreaks has occurred too frequently in the Boreal Forest of North America to be regarded as mere coincidence and it must be concluded that climatic changes are important in the initiation of outbreaks.

The mechanism whereby budworm populations increase rapidly during a period of favorable climate is not fully understood. Data are presented which suggest that fecundity increases and mortality decreases when conditions become optimum for feeding and rapid larval development. As dry and sunny weather promotes larval development, increases in the endemic population may be expected throughout the region that experiences the change to favorable climate. Staminate flowers, which are produced more frequently in a period of dry years, also promote the rate of larval development so that further increases in fecundity can be expected in mature flowering stands. In the present outbreak the first increases were detected in both mature and immature stands. Subsequent increases took place at a far greater rate in extensive stands of mature balsam. Thus the first severe defoliation is generally noticed in mature stands, and it is in such stands that the outbreak gains its momentum. This has led many to report that outbreaks begin in areas of mature balsam fir. Initially, however, climatic release appears to act rather generally but stand factors determine in what areas the infestation will reach a severe stage. The reasons for this are discussed in another paper (19).

The role of climate in the initiation of the outbreaks of the spruce budworm in New Brunswick during this century can be summarized briefly. Weather analysis has shown that regional climatic changes did occur prior to the outbreaks. These favorable climatic changes give strong support to the theory of climatic release and explain the time and place of budworm outbreaks. Dispersal, both from Quebec infestations and within the Province, has played an important role in the course of these outbreaks as will be shown in the second paper.

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EFFECT OF AGE AND PLANE OF NUTRITION ON THE BLOOD CHEMISTRY OF THE COLUMBIAN BLACK-TAILED DEER (ODOCOILEUS HEMIONUS COLUMBIANUS)

A. PACKED-CELL VOLUME, SEDIMENTATION RATE, AND HEMOGLOBIN[1]

By W. D. Kitts,[2] P. J. Bandy,[3] A. J. Wood,[4] and I. McT. Cowan[5]

Abstract

A study has been made of the normal chemistry of the blood constituents of the Columbian black-tailed deer (*Odocoileus hemionus columbianus*) in relation to growth and the caloric plane of nutrition. The results showed a significant difference of packed-cell volume and hemoglobin level between the two age groups of experimental animals, while no significant differences were found in the sedimentation rates. The high and low imposed planes of nutrition did not bring about a significant difference in packed-cell volume, sedimentation rate, or hemoglobin value.

Introduction

The normal and pathological chemistry of the blood constituents of domestic animals in relation to age, breed, type of ration, season, and environmental temperatures have received careful study (1, 3, 7, 9). However, the relationships of the blood elements to the state of nutrition and physiology of wild species has received little attention. A knowledge of such relationships is essential to an understanding of the growth and environmental physiology of such animals. Rosen and Bischoff (11) have reported upon the blood chemistry of several subspecies of *Odocoileus* (*Odocoileus hemionus californicus*, *O. h. columbianus*, *O. h. hemionus*, *O. h. fuliginatus* × *O. h. hemionus*). These workers reported average values for certain blood constituents of the black-tailed deer taken from various Californian herds to be 10.1 ± 0.1 million red blood cells per cu. mm., 16.4 ± 0.17* gm. % hemoglobin, and a packed-cell volume of 44.8 ± 0.5 %. There are indications from their data that near starvation conditions reduced the red cell count and hence the hemoglobin level. They implied that the red cell picture might provide an indicator of environmental adequacy.

Browman and Sears (4) have reported on the blood values of the Rocky Mountain mule deer in Montana. They stated that the blood picture of the mule deer is not radically different from that of domestic livestock sampled under the same environmental conditions. The average values they pre-

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**Computed from per cent hemoglobin values reported by these authors.*

sented are: hemoglobin* 12.8 gm. %, red cell count 9.19 millions per cu. mm., and the packed-cell volume as a percentage of total blood volume, 39.6.

In 1955 Svihla, Bowman, and Pearson (14) reported on the blood picture of the American black bear (*Ursus americanus*). They showed that the average values for red blood cell count, hemoglobin, and packed-cell volume were 17.15 millions per cu. mm., 15.5 gm. %, and 35.3 % respectively during the months of August and September. These values are remarkable in that they show an extremely high red cell count with a relatively low packed-cell volume. This may indicate that the diameter of the red cell in the bear is smaller than that of the ungulates. All the above data refer to wild taken animals of unknown nutritional history. It is interesting to record that the average values for red blood cells, hemoglobin, and packed-cell volume in the ox are 6.3 millions per cu. mm., 12.03 gm. %, and 41.5 % respectively (8, 12).

The certainty with which such field data may be interpreted must rest largely upon the availability of normal values obtained from animals reared under known environmental conditions. Only in this way can the normal changes associated with growth and aging be segregated from those arising from environmental factors.

Studies of growth and nutrition in the Columbian black-tailed deer were initiated by Cowan and Wood in 1949 (5). The present investigation, which is a continuation of this project, was designed to study certain aspects of the normal chemistry of blood constituents in relation to growth and the caloric plane of nutrition.

Methods and Materials

(*a*) *Experimental Animals*

The animals used in this experiment were Columbian black-tailed deer. Two groups of animals were studied; H-group consisting of six deer, born in 1953, which were raised at The University of British Columbia and J-group containing fourteen 1954 fawns that were captured shortly after birth on Vancouver Island and reared at The University of British Columbia.

(*b*) *Feeding*

Both J- and H-group deer were randomly divided into two groups and received two caloric planes of nutrition (2). The high plane group received a calculated maximum intake for optimum growth, whereas the low plane group received 60 % of the caloric intake of the high plane animals. This material will be discussed in detail in a subsequent report.

(*c*) *Blood Samples*

Blood samples of 20 ml. were taken from the recurrent tarsal vein of each deer at frequent intervals between July 5, 1954, and September 23, 1954. The deer blood samples were then flushed into test tubes containing 20 mgm. of potassium oxalate. Samples were then stoppered and stored at 6°C. prior to analysis.

**Computed from per cent hemoglobin values reported by these authors.*

(*d*) *Analytical Procedures*

(*i*) *Packed-cell Volume*

Oxalated blood was centrifuged in hematocrit tubes at 1000 r.p.m. for five minutes. The speed of centrifugation was then increased to 2000 r.p.m. for 15 min. Hematocrit values were recorded and the samples were recentrifuged at 2000 r.p.m. for an additional five minutes. If no change had occurred the final hematocrit values were recorded. If a change was noticed the samples were respun until no further change occurred, and this value was taken as final.

(*ii*) *Erythrocyte Sedimentation Rates*

A slight modification of the procedure of D'Amour and Blood (6) was employed. The deer blood was diluted four parts to one with mammalian Ringer's solution instead of with a 3% solution of sodium citrate.

(*iii*) *Hemoglobin*

The method of Wong (17) was followed without modification.

(*iv*) *Statistical Analysis*

Statistical analysis of the data were made according to Snedecor (13).

Results and Discussion

(*a*) *Packed-cell Volume*

The packed-cell volume is readily measured with a high degree of precision and since it affords the same type of information as does the red cell count or hemoglobin, it has been termed "the most useful single criterion of the degree of anemia or polycythemia at present available(16)". A number of factors such as high altitude, muscular exercise, heightened environmental temperatures, and certain pathological conditions are known to cause an increase in the packed-cell volume. The volume is lowered under conditions of high barometric pressure, low environmental temperatures, and in certain abnormal states.

The current understanding of the change in packed-cell volume with age in normal animals, permits one to anticipate a declining packed-cell volume after birth followed by a slow increase to normal adult levels (10). The high plane H and J deer used in this study may be considered normal as they had instantaneous relative growth rates comparable with those reported earlier as normal for this subspecies (5). Unfortunately, packed-cell volumes could not be measured until 20 days after birth and for this reason it is not possible to state if the deer shows an initial decline in this value as has been reported for man and the pig (16, 10). It is possible, however, from the data in Fig. 1 to discern a gradual rise in packed-cell volume with increasing age as noted for other species. The data presented in Table I, for example, reveal that the 20–100 day age group had a packed-cell volume of 40 % while the 392–465 day age group had a value of 58 %.

The packed-cell volume of H-group deer declined from a level of 58% to 52% between July 12 and September 23 (390–465 days of age) (Fig. 1). This may be a seasonal effect or it may be an indication of the onset of the breeding season. As would be expected, a similar decline in hemoglobin level was noted.

The values cited by other workers (11), for wild taken deer of this subspecies, as well as for *O. h. californicus*, *O. h. hemionus*, and a hybrid between *O. h. fuliginatus* and *hemionus*, are significantly below those of our older age group. The range cited is between 30 and 49%. On the basis of present data these values approximate the change with age in normal deer. On the other hand, animal weights given by Rosen and Bischoff (11) would lead one to believe

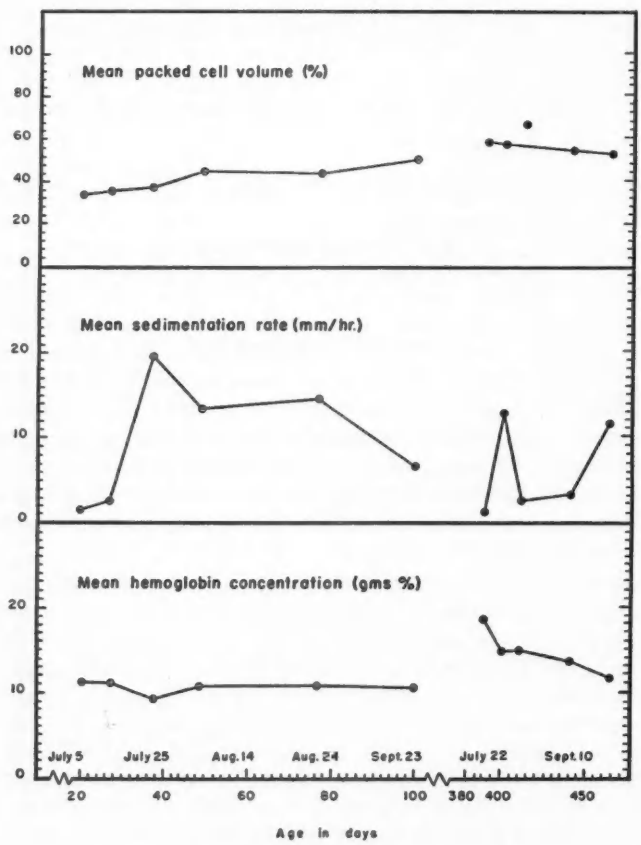

FIG. 1. Changes in the mean values for packed-cell volume, sedimentation rate, and hemoglobin concentration during early growth of the Columbian black-tailed deer. (J-group –O–O–O–O–; H-group –●–●–●–●–.)

TABLE I

HEMATOLOGICAL VALUES OF COLUMBIAN BLACK-TAILED DEER RANGING IN AGE FROM 20 TO 465 DAYS*

(Comparative values of other subspecies of *Odocoileus hemionus* are shown)

Species	Age, days	Packed-cell volume, %				Hemoglobin, gm. %				Sedimentation rate, mm./hr.			
		Mean	S.E.	S.D.	*P*	Mean	S.E.	S.D.	*P*	Mean	S.E.	S.D.	*P*
O.h. columbianus (J)	20–100	40.0	±0.98	8.46	$t = 2.22$	10.70	±0.21	1.78	$t = 9.52$	9.9	±1.42	12.19	$t = 1.48$
O.h. columbianus (H)	392–465	58.2	±1.33	7.02	<0.05	14.93	±0.47	2.49	<0.01	6.2	±1.51	8.02	>0.1
					$n-2 = 100$				$n-2 = 100$				$n-2 = 100$
O.h. columbianus†	Unknown	47.2	±1.5	10.1		15.00	±1.5	16.6					
O.h. californicus†	Unknown	43.5	±0.9	8.1		17.17	±0.6	5.7					
O.h. hemionus†	Unknown	49.1	±1.1	6.0		17.70	±2.6	18.8					
O.h. fuliginatus† × *O.h. hemionus*	Unknown	30.0	±1.8	6.3		13.00	±1.0	3.7					
O.h. hemionus‡	Unknown	39.6				12.8							

*Including both sexes and excluding the influence of the planes of nutrition.

†Rosen and Bischoff (*11*).

‡Browman and Sears (*4*).

that the average packed-cell volumes of the California deer at equal body weight are below those of our series. This may be an expression of environmental influence upon packed-cell volume.

The imposed low plane diet in our H-group deer was so low that growth was virtually at a standstill. The instantaneous relative growth from 360–440 days ranged between 0.0003 and 0.0009 as compared with 0.0013 and 0.0023 for the high plane group for the same period.

In view of the suggested evidence of low packed-cell volume in malnourished wild deer in California (11), it is pertinent to remark that our data reveal (Table II) no such response to the imposed high and low levels of nutrition with either fawns or yearlings. However, it should be emphasized that our deer were below maximum adult body weight and even the low plane animals were still growing slowly. Adult animals starved into serious weight loss might suffer a decrease in packed-cell volume and hemoglobin level. Our experience suggests that a plane of nutrition sufficiently low to alter the packed-cell volume of growing animals might lead to death of the animal before a significant change occurs in the value.

(*b*) *Sedimentation Rate*

Sedimentation rates in the experimental deer varied widely between individuals and between samples from the same individual. The data reveal no significant differences between age groups or between the two planes of nutrition. (See Tables I and II.)

Pathological conditions which have been shown to affect sedimentation rate (8) may be ruled out as causing the variations observed in this experiment. Thus sedimentation rate, as shown in Fig. 1, appears to reflect the changes that occur in packed-cell volume.

(*c*) *Hemoglobin*

Table I shows that there is a significant difference between the hemoglobin levels of the two age groups (J and H), the younger animals having a lower average hemoglobin value than that of the yearlings. This difference may be due in part to the replacement of foetal hemoglobin by the adult form. Thomas *et al.* (15) found the hemoglobin level of dairy calves to be relatively high at birth and to decline shortly after, reaching a minimum value at 40–60 days. As reported by those investigators, the hemoglobin level then gradually increased and reached the adult level during weaning. Fig. 1 suggests a similar decline in hemoglobin value to 37 days post-partum in the Columbian black-tailed deer, followed by an increase to the adult level at 100 days of age.

The data for the yearling deer (H-group) show a peak hemoglobin level to July 12 followed by a decrease from July 22 to August 31. The final value for September 23 reveals a much lower hemoglobin level indicative of some change in the hematopoietic system. Similar changes have been reported for dairy cattle and mule deer by Rusoff *et al.* (12) and Rosen and Bischoff (11) respectively. These investigators attributed the changes in blood levels to changes in environmental temperatures. While this may be so, it is also

TABLE II

THE INFLUENCE OF THE IMPOSED PLANES OF NUTRITION ON THE PACKED-CELL VOLUME, HEMOGLOBIN, AND SEDIMENTATION RATE OF BLOOD FROM TWO AGE GROUPS OF COLUMBIAN BLACK-TAILED DEER

Group	Age, days	Plane of nutrition	Packed-cell volume, %				Hemoglobin, gm. %				Sedimentation rate, mm./hr.			
			Mean	S.E.	S.D.	P	Mean	S.E.	S.D.	P	Mean	S.E.	S.D.	P
J	20–100	Low	39.9	±1.26	7.76	$t = 1.509$	10.19	±0.39	2.44	$t = 1.74$	10.7	±2.34	14.42	$t = 0.528$
J	20–100	High	40.2	±1.55	9.30	>0.1	10.56	±0.42	2.52	<0.1	9.1	±1.79	10.73	>0.5
						$n-2 = 72$				$n-2 = 72$				$n-2 = 72$
H	392–465	Low	57.5	±2.06	7.79	$t = 0.592$	13.75	±0.88	3.40	$t = 0.15$	8.4	±2.51	9.73	$t = 1.551$
H	392–465	High	59.1	±1.64	5.93	>0.1	14.92	±0.94	3.38	>0.5	3.7	±1.29	4.65	>0.1
						$n-2 = 26$				$n-2 = 26$				$n-2 = 26$

possible that the changes in levels of hemoglobin may be associated with hormonal regulations of growth and metabolism preparing for the onset of rut. More work is necessary on this aspect.

Table II shows that no significant differences occurred in hemoglobin levels between high and low plane deer. Since the planes of nutrition represent quantitative differences in caloric intake rather than qualitative difference in dietary constituents, it is suggested that the restriction of the caloric intake to the maintenance level does not affect the rate of hemoglobin formation. The values reported here may thus be regarded as normal, since the caloric plane was not below maintenance and since no specific vitamin or mineral deficiencies were apparent in the experimental animals.

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THE LIFE CYCLE AND SEASONAL TRANSMISSION OF ORNITHOFILARIA FALLISENSIS ANDERSON, A PARASITE OF DOMESTIC AND WILD DUCKS[1]

By Roy C. Anderson

Abstract

White Pekin ducklings kept outdoors in Algonquin Park, Ontario, frequently become infected with *Ornithofilaria fallisensis*. Ducklings exposed during the black-fly seasons in 1952–1955 became infected. The microfilaria of *O. fallisensis* developed to the infective stage in *Simulium venustum* Say, *S. parnassum* Mall., *S. rugglesi* Nicholson and Mickel, and various members of the subgenus *Eusimulium* identified as *S. euryadminiculum* Davies, *S. croxtoni* Nicholson and Mickel, and *S. latipes* Meigen. Only the last four species were found feeding naturally on the ducks in 1955. The microfilaria did not develop significantly in in several species of mosquitoes nor in *Culicoides* sp. Members of the subgenus *Eusimulium* were collected from ducks in 1955 only during the early part of the black-fly season whereas *S. rugglesi* was collected from May 26 to the middle of July. Ducklings exposed when these ornithophilic simuliids were active became infected and a consideration of the times after exposure at which microfilariae appeared in their blood indicates that members of *Eusimulium* were the vectors of *O. fallisensis* in the first part of the fly season and that *S. rugglesi* was the sole vector thereafter. The development of the microfilaria to the infective stage takes place in the haemocoele of the black fly and requires 7–14 days depending on the temperature. There are two molts during development and a "sausage-stage". Microfilariae appeared in the blood of three parasite-free ducklings 30–36 days after third-stage larvae were injected into them; adult worms were recovered from one of these birds. The microfilariae of *O. fallisensis* exhibit a diurnal periodicity. The courses of the microfilaremias of experimentally and naturally infected birds were compared. Re-exposure failed to alter the declining microfilaremias of ducks or to result in a reappearance of microfilariae in ducks from whose blood microfilariae had disappeared. Since parasite-free, adult ducks became infected during exposure, it is concluded that infected birds acquire an immunity to *O. fallisensis*. A few ducklings seemed to exhibit a natural resistance to infection. Adult *O. fallisensis* were found in a black duck (*Anas rubripes*) from Algonquin Park and it is suggested that these birds are the reservoirs of infection in the Park.

Introduction

No fewer than 18 genera of the family Dipetalonematidae Wehr, 1935, are known to occur in birds. It is a remarkable fact, however, that the life cycles of none of these has been elucidated heretofore. Sir Patrick Manson as early as 1878 recorded observations made on the microfilaria of "*Filaria picae mediae*" in the Chinese crow but he apparently made no attempt to elucidate its life cycle (42). Dutton (20), while a member of a mission sent to Gambia, Africa, by the Liverpool School of Tropical Medicine, provided the first information on the life cycle of an avian filarioid. This author described filarioid larvae which he found in the fat bodies of Mallophaga that were infesting swifts (*Cypselus affinis* Gray) in Africa. According to Dutton these lice (subfamily Leiothinae) supplement their feather diet with blood and lymph. He believed that these larval stages belong to "*Filaria cypseli* Annett, Dutton and Elliot" which parasitize the swifts. The microfilariae, according to Dutton, occur in the lymph rather than in the blood and the adults in subcutaneous tissues. Thomas (55), working in Michigan, observed a

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Contribution from the Department of Parasitology, Ontario Research Foundation, and the University of Toronto, Toronto 5, Ontario Canada.

microfilaria, presumably from a domestic duck, in the stomach contents of a freshly killed black fly. He stated that mosquitoes were being tested as possible intermediate hosts. Adult worms were not recovered from an infected adult duck that was dissected. Gönnert (29) undertook experiments, no details of which were given, to determine the vector of *Ornithofilaria mavis* (Leiper, 1909) in a thrush (*Turdus musicus* Linné) but these experiments were unsuccessful. Boughton, Byrd, and Lund (9) observed that microfilariae (species unknown) in a crow (*Corvus brachyrhynchos* Brehm) exhibited a marked nocturnal periodicity, the microfilariae being more abundant in the peripheral circulation when the bird is at rest. They were able to reverse this periodicity by reversing the periods of activity and rest of the birds. Robinson (49) similarly was able to reverse the nocturnal periodicity of microfilariae in the common crow and in the fish crow (*Corvus ossifragus* Wilson). In Michigan, Chernin (14) observed that some of his white Pekin ducks (*Anas boschas* Linné), exposed during July and August, became infected with an unknown filarioid. He believed, as a result of finding microfilariae on stained blood smears, that the prepatent period was from six to nine months. Adult worms were not recovered from infected ducks that were autopsied. Recently Robinson (48) permitted several species of mosquitoes to feed on common crows, blue jays (*Cyanocitta cristata* Linné), a fish crow, white-throated sparrows (*Zonotrichia albicollis* Gmelin), and cardinals (*Richmondena cardinalis* Linné) harboring microfilariae of various undetermined filarioids, but little or no development of the microfilariae took place in these mosquitoes. Robinson, like Jellison (34) earlier, found engorged *Culicoides* in the nests of birds of the family Corvidae and suggested that they might transmit the filarial worms found in crows and jays.

White Pekin ducklings (*Anas boschas* Linné), kept outdoors in the Wildlife Research Area in Algonquin Park, Ontario, during the spring and early summer, frequently become infected with *Ornithofilaria fallisensis* Anderson.* The consistent transmission of this nematode to domestic ducks at the Station offered an excellent opportunity to study in some detail the life cycle and seasonal transmission of an avian filarioid. Accordingly, during the springs and summers of 1953–1955, studies were conducted to determine the vectors of *O. fallisensis*. The author will endeavor to show that the vectors of *O. fallisensis* are blood-sucking flies of the family Simuliidae (order Diptera). In addition to describing the development of *O. fallisensis* in simuliids, the courses of the infections, acquired naturally by domestic ducklings kept outdoors in Algonquin Park in 1952–1955, are described and an attempt is made to correlate these findings with the feeding activity of various ornithophilic simuliids. Ducklings have been infected experimentally with *O. fallisensis* and the courses of their resultant microfilaremias are described and compared with those of birds infected naturally. Observations on the longevity of the infections, resistance of ducks to infection, and periodicity of the microfilariae of *O. fallisensis* are also given.

**Anderson (1) inadvertently gave the scientific name of the domestic duck as Anser domesticus.*

Materials and Methods

During the springs and summers of 1952–55 one- to two-week-old white Pekin ducklings were placed outside in pens in Algonquin Park. Although many of these birds died from *Leucocytozoon simondi* Mathis and Leger and other causes, a few survived each year and these were examined regularly for the presence of microfilariae. Since these ducklings were exposed (viz. placed outdoors) at different times during the spring and summer it was possible to determine roughly the periods during which transmission of *O. fallisensis* occurred. Most ducklings were placed outdoors and left outdoors for the remainder of the black-fly season (continuous exposure) but some were given limited exposures of only 3–14 days after which they were brought indoors for the remainder of the black-fly season. After 1952 various numbers of old ducks, infected with *O. fallisensis* during exposure in previous years, were kept in pens adjacent to those holding the exposed ducklings.

To determine the presence of filarioids in an exposed bird, one drop of fresh blood, removed from the metatarsal vein, was mounted under a vaseline-ringed cover slip and examined for moving microfilariae under the high or medium power of a dissecting microscope.

Examinations were started 30 days after the first day of exposure and were continued until microfilariae were found or until it was concluded that the ducklings were uninfected. Actual counts of microfilariae in known quantities of blood were made in the following way: 6.46 cu. mm. of blood was sucked into a Drummond capillary pipette which had been rinsed previously with heparin. The heparinized blood was then placed on a ruled glass slide and covered with a vaseline-ringed cover slip which was pressed gently to spread the blood uniformly. Most counts were made with the high power of a dissecting microscope.

Ducks infected with *O. fallisensis* were exposed for 20 min. over water or on the edge of the shore of Lake Sasajewan and then placed in flyproof cages for 20 min. Black flies (Simuliidae) which had engorged *naturally* on the ducks were captured by means of aspirators after they came off into the flyproofs. Most attempts to catch flies were made between the hours of 1800–2100 when fly-biting was most frequent, and collections were made throughout the spring and summer of 1955. Flies were captured off ducklings as well to see whether the same species fed on them as on adult ducks.

Certain species of simuliids which do not, or only rarely, feed on ducks were captured in shell vials and allowed to feed on the deplumed heads of infected ducks in the manner described by Fallis *et al.* (23).

Black flies were kept in specially built cages 8 in. wide, 8 in. long, and 9 in. high. One side of the fly cage was covered with muslin, cotton cloth, or silk and the top was covered by a sliding glass plate; the remaining sides and bottom were of wood. One or two armholes with mesh funnels allowed access to the cage. Small cheesecloth bags containing dry sucrose (see Davies (17)) were tacked in parts of the cage frequented by the flies. A damp cloth was placed in the bottom of the cage as well as a jar of water covered with cheesecloth; strips of filter paper acted as wicks, drawing the water to the cheesecloth.

Crevices into which flies could crawl were eliminated. Light was prevented from entering the cage by sheets of black paper attached to the front and top. The sheets of black paper were removed for two to four hours daily during which time the flies were observed to feed and consume water. Up to 200 flies were maintained successfully in a single cage.

The females of many species of black flies are difficult to identify. The writer has been fortunate, however, in having the advice of three specialists namely, Mr. Guy E. Shewell, Division of Entomology, Department of Agriculture, Ottawa, Dr. D. M. Davies, Assistant Professor of Zoology, McMaster University, and Mr. B. V. Peterson of the University of Utah, who confirmed independently most of the author's identifications of representative specimens of black flies used in this study. The identifications of simuliids by the author (Table III) were made with the aid of a key kindly provided by Mr. Shewell (50). Specimens have been pinned and a representative series has been lodged in the Diptera section of the Division of Entomology, Department of Agriculture, Ottawa. Whole specimens, from which third-stage larvae of *O. fallisensis* were removed, were preserved in 95% alcohol and lodged in the Diptera section of the Division of Entomology.

Laboratory-reared *Aedes aegypti* were allowed to feed on the heads of infected ducks which were admitted to the mosquito cage. Several species of wild mosquitoes, notably *Aedes* spp., were captured and allowed to feed on infected ducks in the same way. All the species of mosquitoes, but especially *Aedes* spp., seemed to survive better when allowed to take only partial blood meals. The mosquitoes were maintained, after feeding, in cages similar to those used for black flies and were fed on human blood.

Batches of unidentified biting midges (Ceratopogonidae) were captured in vials and permitted to feed on the deplumed heads of infected ducks. The flies were then transferred to a gallon jar, the mouth of which was covered with bolting silk. They were supplied with water, sucrose, and raisins.

Insects which had fed on infected ducks were examined at intervals for developmental stages of *O. fallisensis*. The flies were dissected in physiological saline and the larvae were transferred to a drop of fresh saline which was subsequently covered with a cover slip ringed with vaseline. Living larvae were studied whenever possible. Third-stage larvae were often fixed in 70% alcohol containing a small amount of glycerine and the alcohol was allowed to evaporate overnight until the specimens remained in pure glycerine, after which they were examined. Permanent mounts of third-stage larvae from most of the species of flies used in this study were made by the methods of Baker (3) and Goodey (30); the former method gave the best results. Larvae were stained occasionally with dilute azur II but it was found that the anatomy of larvae could best be determined in unstained material.

Third-stage larvae, dissected from black flies into physiological saline, were sucked into a syringe and injected subcutaneously into uninfected ducklings. After 20 days the injected ducklings were examined daily for the presence of microfilariae.

Ducklings in which microfilariae were detected following exposure were dissected to confirm the presence of *O. fallisensis*. The worms are difficult to locate, especially in birds which are fat, but the following procedure was reasonably successful. The ducklings were anaesthetized, bled to death, and dissected immediately under strong illumination, usually sunlight. The light striking the inner surface of the skin at an angle is best for it is possible under these conditions to detect movements of the worms which might otherwise be overlooked. Specimens of *O. fallisensis*, recovered from naturally and experimentally infected birds, are in the Helminth Collection of the Ontario Research Foundation.

Results

Natural Infections Resulting from Exposure of Ducklings

Six white Pekin ducks, five of which were exposed in Algonquin Park on June 4 (Duck Nos. 958, 959, 961, 962, 963) and one on June 19 (Duck No. 988) were the only experimental ducks that survived exposure during the spring and summer of 1952. These six birds were kept at Toronto during the fall and winter and blood smears were made from them regularly. Microfilariae of *O. fallisensis* were detected on stained blood smears in October; microfilariae were present in the blood of all six birds at this time.

Ducklings exposed during the spring and summer of 1953 were, unlike the 1952 birds, examined regularly for microfilariae throughout their lives. Because of the distribution of the times that the ducklings were exposed, some indication of the period in which transmission occurred can be obtained. Table I gives the times the ducklings were exposed, the last times they were examined, and the times at which microfilariae were first found in drops of

TABLE I

Results of exposing ducks in Algonquin Park, 1953–54

1953					1954				
No.	Exposed	Last exam. (days)	Mf.	Leuc.	No.	Exposed	Last exam. (days)	Mf.	Leuc.
1100	May 12–June 2	115	–	May 25	1465	May 12–18	82	–	–
1101	May 12–June 2	115	–	May 25	1466	May 12–18	82	–	–
1109	May 27–June 7	100	–	June 6	1467	May 12–18	82	–	–
1110	May 27–June 7	100	–	June 4	1468	May 12–18	82	–	–
1111	May 27–	59	+	June 4	1477	May 20–26	74	–	–
1113	May 27–	59	+	June 5	1478	May 20–26	74	–	–
1116	June 3–	48	+	June 9	1481	May 29–	50	+	June 7
1118	June 3–	50	+	June 9	1482	May 29–	57	+	June 7
1119	June 3–	50	+	June 9	1484	May 29–	53	+	June 7
1136	June 15–	52	+	June 22	1491	June 4–	45	+	June 10
1142	June 16–	57	+	June 22	1496	June 4–	47	+	June 12
1328	July 23–	54	–	No data	1508	June 15–17	37	+	June 26
1326	July 24–	53	–	No data	1608	July 16–20	95	–	July 21
1331	July 24–	53	–	–	1609	July 16–20	51	–	July 21
1371	July 24–	53	–	–	1631	Aug. 1–	79	–	Aug. 12
1372	July 24–	53	–	–	1643	Aug. 3–	85	–	Aug. 12
1377	July 24–	53	–	–	1626	Aug. 12–	76	–	No data
1381	July 26–	51	–	July 31	1627	Aug. 12–	76	–	No data

blood from their metatarsal veins. The last column, recording the incidence of *Leucocytozoon simondi* in the ducklings, will be dealt with later. Table I shows that microfilariae were not detected in the blood of two birds exposed from May 12 to June 2 or in another two birds exposed from May 27 to June 7. These four birds were the only ones that were exposed for a limited time only, the remainder being left outdoors. Seven birds exposed continuously for periods beginning from May 27 to June 16 all became infected with *O. fallisensis*. Microfilariae were first detected in drops of blood from these birds 48–59 days after the first day of exposure. The periods between exposure and the appearance of microfilariae were longest in two birds (1111, 1113) whose exposure periods started in late May and ended in early June when transmission apparently did not occur, as indicated by the fact that birds (1100, 1101, 1109, 1110) given limited exposures from May 12 to June 7 remained uninfected. There is, unfortunately, a gap in the data from June 16 to July 23 because all ducks exposed during this period died from the effects of *Leucocytozoon simondi* and other causes before microfilariae could be expected to appear in their blood. Microfilariae were not detected in the blood of birds exposed from July 23 onwards. It was not possible to prolong the examination of these latter ducks beyond the times indicated in Table I (51–54 days) since they were killed by wild mink. It is conceivable that some of these ducks had light infections which would not be expected to show up as quickly as in those birds exposed earlier, e.g. June. However, the times at which they were last examined are similar to those of the infected birds and it can probably be concluded that, if transmission did occur during this period, it was less pronounced than during the month of June.

Essentially the same picture was presented in 1954 as in 1953, although the data are again fragmentary because many of the ducks died, particularly during July (Table I). There was no evidence of transmission up to May 26 or during August, all the birds which became infected being exposed on May 29 or in June. Apart from the birds exposed from May 12–26, only three birds given limited exposures survived and of these one (1508) was exposed from June 15–17 and two (1608, 1609) from July 16–20; microfilariae were detected only in the bird exposed during June. Microfilariae were first detected in the blood of the birds which became infected, at times similar to those exposed in 1953, although they were detected in one bird (1508) 37 days after it was exposed.

During the spring and summer of 1955 fewer ducks died from the effects of *L. simondi* and other causes than in previous years and a more detailed picture of the transmission of *O. fallisensis* was obtained (Table II). All the ducklings were exposed continuously from the times they were first exposed. Microfilariae were detected in the blood of infected birds 36–88 days after they were first exposed and they took longest to appear in ducklings exposed first on May 9. Ducklings exposed during the last week of June and during the first week in July also became infected; in other years none of the ducklings, exposed during this period, survived for a sufficient length of time

TABLE II

RESULTS OF CONTINUOUS EXPOSURE OF DUCKLINGS IN ALGONQUIN PARK, 1955

Duck No.	Exposed	Last exam.	Total days exam.	Results
1659	May 9	July 10	62	+
1660	May 9	Aug. 5	88	+
1664	May 18	July 11	55	+
1666	May 18	July 12	54	+
1674	May 29	July 10	42	+
1676	May 29	July 8	40	+
1682	June 4	Aug. 2	59	+
1686	June 4	July 11	37	+
1687	June 4	Aug. 15	72	−
1692	June 4	July 15	41	+
1693	June 4	July 14	40	+
1694	June 4	July 10	36	+
1703	June 14	July 22	38	+
1706	June 14	Aug. 18	65	+
1711	June 14	Aug. 10	57	+
1712	June 14	July 22	38	+
1713	June 14	Aug. 10	57	+
1732	June 26	Aug. 24	59	−
1735	June 26	Aug. 12	47	+
1737	June 26	Aug. 16	51	+
1739	June 26	Aug. 9	44	+
1740	June 26	Aug. 6	41	+
1741	June 26	Oct. 1	99	−
1743	July 2	Aug. 10	39	+
1744	July 2	Oct. 1	54	−
1746	July 2	Aug. 10	39	+
1758	July 9	Oct. 3	86	−
1759	July 9	Oct. 3	86	−
1764	July 9	Oct. 3	86	−

to establish whether transmission occurred. Microfilariae were not detected in four of the 26 ducklings exposed between May 9 and July 2. Microfilariae were not detected in the blood of one duckling exposed on July 2 nor in those exposed on July 9.

SIMULIIDAE AS HOSTS FOR THE LARVAL STAGES OF *Ornithofilaria fallisensis*

During the spring and summer of 1955 black flies were caught after they had fed naturally on exposed ducks and ducklings. Some of these, collected from infected ducks, were kept in cages and examined later for larval stages of *O. fallisensis*. Regular catches of flies from ducks and ducklings yielded *Simulium* (*Eusimulium*) *croxtoni* Nicholson and Mickel, *S.* (*E.*) *euryadminiculum* Davies, *S.* (*E.*) *latipes* Meigen, and *S.* (*Simulium*) *rugglesi* Nicholson and Mickel. The same species fed on ducklings as those fed on ducks and the combined data are given in Table III. Species of the subgenus *Eusimulium* were first observed feeding on the ducks on May 21. However, these flies must have been active before this date. Gametocytes of *Leucocytozoon*

TABLE III

SUMMARY OF FLIES COLLECTED FROM ADULT AND JUVENILE DUCKS, ALGONQUIN PARK, 1955

Date	*croxtoni*	*euryad.*	*latipes*	*rugglesi*	Undet. *Eusim.*
May 21	40	14	10	0	2
May 22	39	17	19	0	2
May 26	54	9	28	22	2
May 27	6	2	2	3	0
May 28	1	2	1	28	0
May 29	7	4	12	23	2
June 2	8	5	6	22	1
June 3	17	2	2	2	2
June 5	0	0	0	7	0
June 6	15	8	5	13	0
June 7	3	1	1	3	0
June 9	0	0	1	4	0
June 10	0	0	0	51	1
June 11	0	0	0	6	0
June 14	0	0	0	8	0
June 15	0	1	0	71	0
June 16–July 10	0	0	0	290	0

NOTE: *Flies were collected from varying numbers of ducks depending on what use was to be made of them. Thus the data cannot be interpreted quantitatively to indicate the relative number of flies biting at various times. Flies were captured 18 evenings between June 16 and July 10.*

simondi appeared in the blood of ducklings on May 27 and as the prepatent period of *L. simondi* is given as about six days (23) it can be concluded that flies which had previously fed on birds were active on May 21. Since *L. simondi* requires an estimated four days to reach the infective stage in the flies, at this time of year, it is probable that some flies were biting at least by May 17. This estimate is supported by the fact that, although no flies were observed feeding on the birds before May 21, *S. euryadminiculum* was first collected by the author in sweeps on May 15. *Simulium rugglesi* was first collected from ducklings on May 26. Members of the subgenus *Eusimulium* were not collected from ducks or ducklings after June 15 but *S. rugglesi* was collected up to July 10. *S. rugglesi*, or some other undetected simuliid, was undoubtedly active after this date as some of the ducklings exposed as late as July 25 became infected with *L. simondi* (22).

It has been found that *O. fallisensis* will develop to the infective stage in all the above-mentioned simuliids. Unfortunately at the time these ornithophilic simuliids were being investigated, a duck, heavily infected with *O. fallisensis*, was not available. It was necessary, therefore, to catch and maintain flies which had engorged on birds with light infections. Thus the number of flies which became infected from feeding on the ducks was low and few larvae were obtained from those flies which became infected (cf. the results, given below, when *S. venustum* were fed on a heavily infected duck). Nevertheless the writer has dissected seven *S. croxtoni*, four *S. euryadminiculum*, five *S. latipes*, and 11 *S. rugglesi* which contained third-stage larvae a few days after they had engorged on infected ducks. Third-stage larvae were found

in the mouth parts of all these species of flies. In addition, three *S. croxtoni*, six *S. euryadminiculum*, one *S. latipes*, and eight *S. rugglesi* that were dissected after feeding on infected ducks contained second-stage larvae which presumably would have developed into third-stage larvae. Earlier larval stages were found in many more flies but these observations will not be detailed. One *S. rugglesi*, which was caught in the field during 1955, contained third-stage larvae which were indistinguishable from those of *O. fallisensis*.

S. rugglesi and the members of the subgenus *Eusimulium* mentioned above crawl under the feathers and down of ducks and ducklings easily by moving against the grain of the feathers. *S. croxtoni*, *S. latipes*, and *S. euryadminiculum* seem to prefer to crawl under the feathers of the back and neck; they are readily seen on the ducks for this reason. *S. rugglesi*, on the other hand, is more difficult to observe as it crawls under the feathers close to the water level or, if the bird is out of water, the feathers near the legs; the latter observation agrees with the report of Shewell (51) on the feeding habits of this fly. Its ornithophilic habits were first suggested by the observations of Dr. A. M. Fallis in 1954, who found it feeding naturally on ducks in Algonquin Park and showed that it was a suitable host for *L. simondi*. It is interesting to note, moreover, that all the above species possess the exaggerated basal claw which Shewell (51) had shrewdly suggested may aid in penetrating feathers.

Simulium (*Simulium*) *venustum* Say is the most prevalent anthropophilic species of black fly reported in Algonquin Park (16). O'Roke (44, 45, 46) reported that this species is the vector of *L. simondi* in Michigan. The specific identity of the black fly reported by O'Roke to be the vector of *L. simondi* has recently been questioned by Shewell (51), who suggests that the black fly observed by O'Roke was *Simulium rugglesi* rather than *S. venustum*. It was important therefore to determine whether *O. fallisensis* will develop in *S. venustum* and, if so, whether this fly feeds naturally on ducks. Accordingly three large batches of *S. venustum* were captured in shell vials and allowed to feed under vials on the deplumed head of a heavily infected duck. The results of dissecting these flies show that *S. venustum* is a highly suitable host for the larval stages of *O. fallisensis* (Table IV). The suitability of

TABLE IV

EXAMINATIONS FOR LARVAE OF *Simulium venustum* FED PREVIOUSLY ON DUCKS INFECTED WITH *O. fallisensis*

Expt. No.	8	11	15
Flies fed	198	191	111
No. days	13	9–13*	11
Flies alive	39	75	20
No. with larvae	34	59	13
No. with 3rd larvae	31	48	9
3rd larvae in head	233	595	20
3rd larvae in thorax	63	113	0
3rd larvae in abdomen	151	200	8
Total 3rd larvae	447	908	28

**Fifteen flies examined at nine days; 15 at 11 days; remainder at 13 days.*

S. venustum as an intermediate host is further emphasized by the almost complete lack of host reaction about the larvae even in flies with heavy infections.

Although *S. venustum* is an excellent experimental intermediate host and can readily be induced to vial-feed on ducks, it does not appear to be a common biter of ducks. During the spring and summer of 1955 only three *S. venustum* were collected while they were feeding naturally* on fully feathered ducks. On May 21 two *S. venustum* were collected from ducklings; one was feeding under the down on the neck, the other under the down on the back. Considerable effort was required to dislodge the flies without damaging them. On the same date a single *S. venustum* was collected as it fed beneath the eye of a sick and inactive drake. The ducks, mentioned above, were kept in pens about 20 feet from the shore of Lake Sasajewan, Algonquin Park. Notwithstanding these observations, engorged *S. venustum* were not caught along with the *S. rugglesi*, *S. croxtoni*, *S. latipes*, and *S. euryadminiculum*, which emerged from the feathers of ducks placed in flyproof cages, although this species was often exceedingly abundant and troublesome to the workers collecting flies. Thus the prognostication of Shewell (51) that *S. venustum* does not usually feed on ducks seems to be borne out. *S. venustum* is very broad in its selection of animals on which to feed (18) and in view of the ease with which it can be vial-fed (23) it does not appear to have any great aversion to feeding on ducks. The writer has often seen *S. venustum* alight and begin feeding on the deplumed heads, or around the bases of the bills, of ducks which were immobilized. In one instance, two *S. venustum* were collected as they fed on the deplumed head of an adult duck which had been placed in a cage used for holding the birds under the flyproof cages. It is possible, therefore, that *S. venustum* was not found feeding on ducks more often because it is incapable, under most conditions, of penetrating duck feathers.

Both in 1954 and 1955 third-stage larvae of *O. fallisensis* were found in *Simulium* (*Simulium*) *parnassum* Malloch which had been vial-fed on infected ducks. Eighteen individuals were kept alive for four to seven days after feeding. Seven of these were positive for larval stages when dissected and the heads of four contained third-stage larvae. No evidence was found, nor is any such recorded (18), however, to indicate that this species ever feeds naturally on ducks.

Prosimulium hirtipes (Fries) was tested both in 1954 and 1955 to determine whether the microfilariae of *O. fallisensis* would develop in it. Frequently mixtures of *P. hirtipes* and *S. venustum* were vial-fed on infected ducks and it was possible to compare the development of the ingested microfilariae in the two species of flies. Infective or second-stage larvae were generally found in most of the *S. venustum* a few days after they had fed. However, only "sausage-stage" larvae (thickened, early, first-stage larvae) were found in a few of the 21 *P. hirtipes* which were alive after feeding at times that were comparable to those of the *S. venustum*. It has been concluded therefore

**As distinct from vial-feeding or feeding on birds which were immobilized.*

that *P. hirtipes* is not a suitable intermediate host for *O. fallisensis*. Moreover, *P. hirtipes* was not observed feeding naturally on healthy active ducks or ducklings although the author saw one alight and begin feeding below the eye of a duck which was being held.

Cnephia (*Mallochianella*) *mutata* (Malloch), *Simulium* (*Neosimulium*) *vittatum* Zetterstedt, and *S.* (*Simulium*) *decorum* Walker were collected around the duck pens in 1955 but none was observed feeding on ducks.

Experimental Transmission of Infective Larvae

It was necessary to establish whether third-stage larvae, recovered from black flies, would produce infection in parasite-free ducklings and also how soon microfilariae could be detected, by the methods used in this study (examining daily a fresh drop of blood from the metatarsal veins), in ducklings which had received known numbers of larvae. Accordingly third-stage larvae, recovered from *S. venustum*, were injected subcutaneously into three two-week-old ducklings. Duckling 1502 received 371 larvae over a period of two days, duckling 1503, 737 larvae over a period of four days, and duckling 1562, 68 larvae over a period of two days. The larvae injected into 1502 were obtained from *S. venustum* used in Experiment No. 8 and those injected into 1503 were obtained from *S. venustum* used in Experiment No. 11 (see Table IV). Larvae injected into 1562 were obtained from Experiment No. 15, the results of which are also summarized in Table IV, and other miscellaneous groups of *S. venustum* and *S. parnassum* that will not be directly referred to. The injected ducklings were kept indoors in flyproof cages throughout the course of this experiment and starting on the 20th day after they were injected, a drop of blood was removed from the metatarsal vein daily and examined for moving microfilariae.

Microfilariae were first detected in the blood of duckling 1502, 30 days after larvae were inoculated into it. In duckling 1503 microfilariae were detected 32 days after it was inoculated and in duckling 1562, 36 days after it was inoculated. Duckling 1502 was killed 105 days after it was injected and adult specimens of *O. fallisensis* were found beneath its skin. The microfilaremias of these birds were followed and these data will be presented in a later section when they will be compared with similar data from ducklings infected naturally while they were exposed in Algonquin Park.

Relationship of Black-fly Activity to the Incidence of Infection with *O. fallisensis*

It has been shown above that various members of the genus *Simulium* feed on ducks and are suitable intermediate hosts for *O. fallisensis;* but can these flies be considered as vectors of this filarioid ? It is believed by the author that a consideration of the periods of activity of these ornithophilic simuliids and the times after exposure at which microfilariae were first detected in the blood of ducklings, exposed in Algonquin Park, will show that members of the

subgenus *Eusimulium* (*S. croxtoni*, *S. euryadminiculum*, and *S. latipes*) and *S. rugglesi* acted as vectors of *O. fallisensis* in 1955, the former in late May and perhaps early June, the latter from about June 5 onwards. In addition, the information obtained in 1955 can be used to explain, in part, the transmission of *O. fallisensis* to domestic ducklings in previous years in Algonquin Park.

Two major factors might be expected to have influenced the times after exposure at which microfilariae were first detected in drops of blood from the ducklings that were infected naturally in Algonquin Park, namely, (1) the biting, during exposure, of ducklings by flies containing infective larvae and (2) the number of larvae received by the ducklings during the first day or two on which they were bitten by flies containing infective larvae. Some ducklings were exposed for several days before black flies were active and thus the times after exposure at which microfilariae were first detected in their blood were unusually long. Moreover, transmission of *O. fallisensis* would only become possible after the parasite has become established in the black-fly populations and has had sufficient time to reach the infective stage in the flies (this may require nearly two weeks; see below). Thus the appearance of flies on the ducklings early in the black-fly season would not necessarily indicate that *O. fallisensis* was being transmitted; this consideration is particularly important in interpreting the incidence of infection in ducklings exposed early in the black-fly season.

The second factor—the number of larvae received by ducklings during the first day or two of exposure to flies containing infective larvae—is important because the first microfilariae detected in the blood of an exposed duckling must be, generally speaking, the progeny of the adult phase of the first infective larvae received. If only a few infective larvae are received initially by an exposed duckling, there will be few adult worms to produce microfilariae and accordingly it will take longer for microfilariae to build up to detectable numbers in the blood. This reasoning is supported by the fact that microfilariae were detected earlier (30–32 days) in the blood of ducklings into which large numbers of larvae were injected than in the duckling which received only 68 larvae (36 days). Undoubtedly many exposed ducklings became infected on the first day that they were exposed. Yet microfilariae were detected in 37 days or less in only three of the 35 ducklings which were infected during exposure in 1953–1955, viz. duckling 1508 (1954)—37 days; 1686 (1955)—37 days; 1694 (1955)—36 days. It is considered unlikely that many exposed ducklings would receive as many as 68 larvae on the first day of exposure, and it is believed that this is the main reason why microfilariae were not detected earlier than the 36th day in ducklings exposed to flies which could be expected to harbor third-stage larvae. The prepatent period of *Litomosoides carinii* (Travassos, 1919) in the cotton rat may be 70 days rather than the usual 50–60 days if only one mature female worm is present (38). Allowing for variations in the sizes of the ducklings and in the sizes of the blood samples that were examined for microfilariae, it seems

reasonable to assume that exposed ducklings, in which microfilariae were detected in less than 40 days after exposure, were initially infected with third-stage larvae on the first day or two on which they were exposed. The following conclusions are based, in part, on this assumption.

It is probable that five ducklings exposed during May, 1955 (1659, 1664, 1666, 1674, 1676, see Table II) were initially infected through the agency of members of the subgenus *Eusimulium* which were shown above to be active about May 17. The development of the microfilaria of *O. fallisensis* to the infective stage in these flies would probably require at least 12 days at this time of year since the average of the temperatures recorded every two hours between 1200 hr. on May 17 and 1200 hr. on May 29 was 56.8° F. (see following section on the development of *O. fallisensis*, especially Table V). Therefore, flies harboring infective larvae could be expected around May 29. Fortunately two of the ducklings (1674, 1676) were exposed on May 29 (Table II) and microfilariae were first detected in their blood 42 and 40 days later indicating that they first became infected within a day or two of exposure. Similarly microfilariae were first detected, in the blood of ducklings 1659, 1664, and 1666, 42, 44, and 43 days respectively after May 29 indicating that they were first infected between May 31 and June 2. Thus microfilariae were first detected in the blood of all five birds at times after exposure that are compatible with the known feeding activity of members of the subgenus *Eusimulium* and the times that infective larvae would be expected in the flies of the subgenus *Eusimulium*.

The times that microfilariae were first detected in the blood of the above-mentioned ducklings do not agree as well with the activity of *S. rugglesi* as they do with the period of activity of members of the subgenus *Eusimulium*. *S. rugglesi* was first observed feeding on the ducks on May 26. It was cool and rainy on May 23–25 and there was no indication that simuliids were feeding on the ducks during these days. Thus it can be assumed with reasonable confidence that *S. rugglesi* first started to feed on the exposed ducks and ducklings on May 26. The development of the microfilaria of *O. fallisensis* in *S. rugglesi* would probably require about 10 days at this time of year since the average of the temperatures recorded every two hours between 1200 hr. on May 26 to 1200 hr. on June 6 was 58.2° F. (see following section on the development of *O. fallisensis*, especially Table V). Therefore, if *S. rugglesi* first fed on the ducks on May 26, it is doubtful whether it would have been capable of transmitting *O. fallisensis* until June 5. However, microfilariae were first detected in the blood of the ducklings at least a week sooner than one would expect if they had first become infected on June 5. Microfilariae were detected in the blood of ducklings 1659, 1664, 1666, 1674, and 1676, 35, 34, 36, 35, 33 days respectively after June 5. It has already been mentioned that it is unusual for microfilariae to be detected in the blood of ducklings, infected during exposure, before 37 days; in fact microfilariae were detected before 37 days after exposure in only one of the 35 naturally infected ducklings used in this study, viz., duckling 1694 (1955) in which microfilariae were detected in

36 days. The most reasonable conclusion seems to be, therefore, that the five ducklings became infected considerably (at least a week) earlier than June 5 and probably before *S. rugglesi* was capable of transmitting infective larvae.

Ducklings exposed on June 4, especially 1686 and 1694, in which microfilariae were detected in 37 and 36 days respectively after they were exposed, may have been first infected through the agency of various species of the subgenus *Eusimulium* which were still feeding on the ducks in some numbers at that time. It is also possible, however, that some of the microfilariae first detected in these ducklings were the progeny of adult worms received as infective larvae from *S. rugglesi* since this fly was, as noted above, probably capable of transmitting larvae about June 5. Ducklings exposed for the first time after June 14 were undoubtedly infected through *S. rugglesi* since, except for a single *E. euryadminiculum*, only this species was collected from ducks or ducklings after this date (Table III).

Fallis *et al.* (22) have recently shown that members of the subgenus *Eusimulium* and *S. rugglesi* transmit *Leucocytozoon simondi* to ducklings in Algonquin Park. Since the prepatent period of *L. simondi* in ducklings and the approximate time required for development to be completed in the black fly are known, one can estimate the time at which black flies first started to feed on exposed ducks and ducklings by noting when gametocytes of *L. simondi* first appeared in the blood of exposed ducklings. Thus, although no attempt was made to collect black flies from ducks or ducklings in 1953 or 1954, one can, nevertheless, obtain some understanding of the feeding activity of these flies in these years. Table I includes columns stating when gametocytes were first noted in the blood of the ducklings exposed in 1953 and 1954.

In 1953 gametocytes of *L. simondi* were first found in the blood of exposed ducklings on May 25 (Table I). Since the development of *L. simondi* in the black fly may be completed in about four days at this time of year, and since the prepatent period in the ducklings may be seven days or less (23), it is possible that black flies first started to feed on the ducks about May 14. It is difficult to understand, therefore, why the four birds (1100, 1101, 1109, 1110), given various limited exposures between May 12 and June 7, did not become infected with *O. fallisensis* (a possible explanation will be offered in the discussion at the end of this paper). Nevertheless the data suggest that *O. fallisensis* was not transmitted until after June 7. If the birds exposed continuously from May 27 (1111, 1113) did not become infected until on or after June 8 then microfilariae were detected in their blood at a maximum of 47 days after they were exposed to flies which might have harbored infective larvae. Similarly the three birds exposed on June 3 (1116, 1118, 1119) may have become infected first on or after June 8 in which case microfilariae were detected in their blood at a maximum of 43, 45, and 45 days respectively after they were exposed to flies harboring infective larvae. These revised times at which microfilariae were detected in the ducklings are actually more in agreement with the data obtained in 1955 than are the unrevised times; the average time that microfilariae were first detected in birds exposed *after* May

29 in 1955 was 45 days. It should be stressed here, however, that in these considerations of the data for 1953 as well as those to follow, the writer does not mean to imply that each duckling became infected on the first day that flies harboring infective larvae were active. He suggests only that the ducklings could not have become infected until such flies were active and that by taking this fact into consideration it is possible to arrive at figures which more accurately represent the time required for microfilariae to appear in the blood of naturally infected ducklings.

In 1954 gametocytes of *L. simondi* were first found in the ducklings (1481, 1482, 1484, Table I) on June 7 indicating, by the same reasoning used above, that flies were first feeding on the ducks around May 27. This estimate is strongly supported by the fact that ducklings (1465, 1466, 1467, 1468, 1477, 1478) exposed from May 12–26 did not become infected with *L. simondi.* Assuming that flies first fed on the ducks on May 27 and that development of *O. fallisensis* to the infective stage would require about 10 days, then the first flies harboring infective larvae would be expected around June 6. If so, microfilariae were detected in the blood of the ducklings exposed on May 29 (1481, 1482, 1484) at a maximum of 42, 49, and 45 days respectively after June 6 when flies harboring infective larvae may have first been active. Similarly the ducklings exposed on June 4 (1491, 1496) were possibly not infected until June 6 or later in which case microfilariae were detected in their blood 43 and 45 days respectively after the date when flies harboring infective larvae would be expected. The revised times that microfilariae were first detected in the blood of the ducklings are remarkably similar to the corresponding times obtained in 1955.

Development of *O. fallisensis* in the Simuliidae

In addition to establishing several species of the Simuliidae as vectors or experimental intermediate hosts of *O. fallisensis*, the development of the microfilaria, after it was ingested with the blood meal of the black fly, was studied. The morphological changes that take place during the development of the microfilaria to the infective, third-stage larva were determined from numerous larvae dissected from some 198 flies, consisting of 105 *Simulium venustum*, 11 *S. parnassum*, 30 *S. rugglesi*, nine *S. latipes*, 17 *S. euryadminiculum*, and 26 *S. croxtoni*, that had fed on infected ducks. Development of the larvae was similar in all these flies and the following account is based on studies of larvae removed from all of them.

The larvae of *O. fallisensis* usually develop in the haemocoele of the abdomen. Developing larvae were occasionally dissected from the head and thorax, presumably in the haemocoele of these regions, but, as far as could be determined, few, if any, of these developed past the very early stages. To determine the route taken by the microfilaria in its journey to the haemocoele, the alimentary canals of several *S. venustum* which had engorged on an infected duck a few minutes previously were removed intact and examined after they were placed in physiological saline in depression slides. By watching the wall

of the distended stomachs through the high power lens of a dissecting microscope, it was possible to see, on three occasions, microfilariae appear on the outer surfaces of the stomach walls and wriggle into the surrounding saline. It is concluded, therefore, that the microfilariae reach the haemocoele by penetrating the stomach wall. This conclusion is supported by the fact that microfilariae found in the abdominal cavities of the flies a few minutes after feeding were always found in the region of the stomach. Dissections of flies at various times after they had engorged on infected ducks showed that some microfilariae penetrated the stomach wall as early as 15 min. after they had been ingested.*

During development of the microfilaria there was first a decrease in length and an increase in diameter accompanied by a marked decline in activity. After this short form ("sausage-stage") was reached, there was rapid growth, involving two molts, which terminated in the infective, third-stage larva. The changes in the dimensions of the developing larvae were studied in a batch of *S. venustum* which had fed on an infected duck (Expt. No. 12). Starting 36 hr. after the flies had fed, two flies were dissected from this batch of flies daily and the developing larvae found in them were measured. The lengths and widths of these larvae, from microfilaria to the third-stage, are plotted in Fig. 1 (each point is the average of measurements of 10–16 larvae). The volumes of the above-mentioned larvae were calculated from the same data used in text Fig. 1 after assuming that the larvae were essentially cylindrical in shape throughout the course of their development. The increase in volume, as determined by these calculations, indicates that the growth of the larvae can be illustrated by a typical sigmoid curve (Fig. 2). The larvae were not exactly cylindrical in shape, however, especially during the first day or two of development. Therefore, the calculated volumes are somewhat larger than they should be. To offset this somewhat, the curve in Fig. 2 is drawn beneath the calculated volumes of the larvae which had developed for 36 or 48 hr. The variations from the cylindrical shape in the microfilariae and in stages after two days of development in the fly are not considered great enough to alter the basic shape of the curve.

The experimental flies were kept in an unheated laboratory where the temperatures approximated those outdoors. To obtain some indication of the rate of development at the various temperatures during the latter part of May, the first three weeks in June, and the first week in July, the averages of the temperatures, recorded every two hours, were calculated for the periods during which development of larvae was taking place in five different batches of *S. venustum*. The temperatures were taken from a thermograph kept in a Stevenson screen outside the laboratory. These average temperatures and the times at which the experiments were begun, molting was first observed, and the third-stage larvae were first found in the head are given in Table V. The time taken for the larva to reach the infective stage was considerably shorter

**The microfilaria of O. fallisensis is enveloped by a delicate sheath which, by analogy with Wuchereria malayi (25), is presumably shed in the stomach of the fly. Attempts to observe the shedding of this exceedingly transparent membrane were not successful however.*

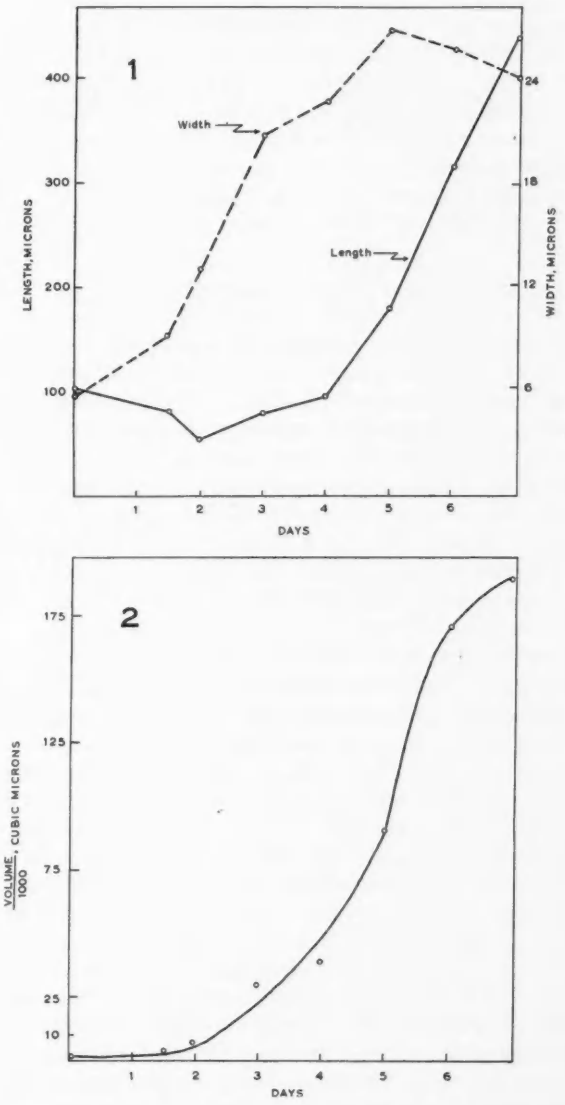


FIG. 1. Changes in the dimensions of developing larvae of *Ornithofilaria fallisensis*.
FIG. 2. Changes in the volume of developing larvae of *Ornithofilaria fallisensis*.

TABLE V

DAYS REQUIRED FOR DEVELOPMENT OF *O. fallisensis* IN *Simulium venustum* AT DIFFERENT OUTDOOR TEMPERATURES

Experiment No.	Start of experiment	Days until 1st molt	Days until 2nd molt	Days until migration to head	Average* temp., °F.
7	May 25, 1954	8	12	14	54.2
2	May 21, 1955	—	9	10	58.4
13	June 20, 1954	5	6	7	62.0
26	July 6, 1955	4	—	7	62.5
12	June 15, 1954	5	7	8	62.8

**Average of temperatures taken at two-hour intervals during experiment.*

during June and July than it was during the latter part of May. However, there may be considerable variation in the time required during late May, depending on the temperature. The shortest time in which infective larvae were found after microfilariae were ingested was seven days. In Expt. No. 12 infective larvae were first found in the heads of the flies on the eighth day although the average temperature was slightly higher than in other experiments in which development was completed in seven days. This difference is probably not significant as there is some variation in the rate of development apart from that due to temperature. As noted earlier, the time required for development has a bearing on the transmission of *O. fallisensis* in the field.

Development to the First Molt—The First Stage Larva

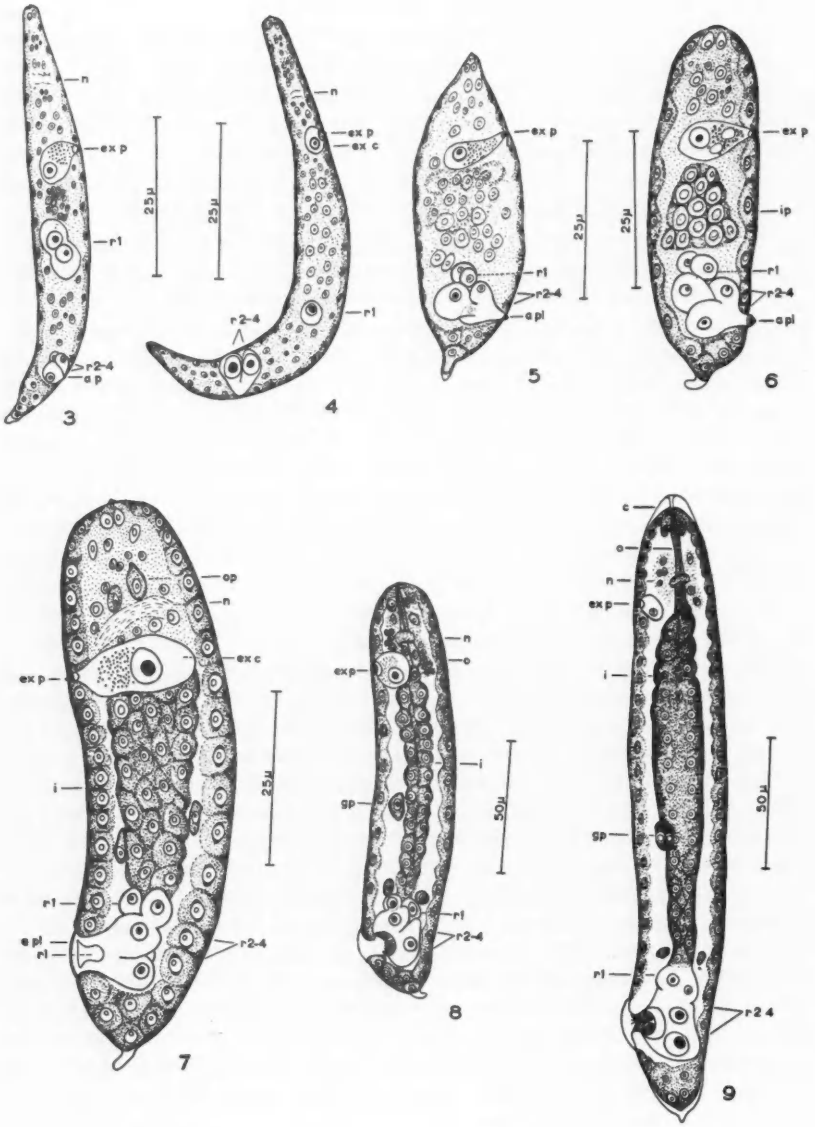
The first indication of development of the microfilaria (see Anderson (1) for the undifferentiated microfilaria) was a pronounced thickening of the body especially marked in the posterior half (Fig. 3). This thickened form became quiescent in contrast to the microfilaria, which was always wriggling. Soon after the body had thickened the rectal cells ("genital cells"), especially the posterior three (*r* 2–4), enlarged. No changes were noted in the cells of the nuclear column at this time. The inner body could not be seen. A few hours later each of the *r* 2–4 cells assumed the shape of a droplet with its pointed end towards the anal pore. Then the pointed extremities of these cells coalesced behind the anal pore and a tripartite cluster of cells was formed (Fig. 4). The larva gradually shortened and when it was about 60 μ long the first rectal cell (*r* 1) divided; the resultant daughter cells remained more or less in tandem just behind the middle of the body (Fig. 4). The excretory cell enlarged and granules began to appear in its cytoplasm. Little change occurred in the cells of the nuclear column at this time although in some specimens the cells in front of the divided *r* 1 cell seemed to cluster together (Fig. 4).

A few hours later the cells of the nuclear column increased in size. This was accompanied by a further thickening and shortening of the body especially in the middle region. At this time the larva measured about 40–50 μ in

length and had a maximum diameter of about 18 μ (Fig. 5). Much of the contraction of the body took place in the region between the *r* 1 cells and the *r* 2–4 cells with the result that the former came to lie immediately above the latter (Fig. 5). The excretory cell increased in length and extended two-thirds of the breadth of the larva in the region of the excretory pore. A number of cells behind the excretory pore grouped together at this time and they formed a faintly granular mass—the primordium of the future intestine. The muscle cells were exceedingly narrow and they formed a thin, delicate lining to the cuticle. The anal pore was marked by a tiny, refractory protuberance continuous with the attenuated ventral ends of the *r* 2–4 cells.

Thickening of the larva continued, especially at the extremities, until it was of more or less uniform diameter from the anterior end to the anal pore (Fig. 6). This particular stage is appropriately termed the "sausage-stage" by most authors. At the same time, however, the larva was increasing slowly in length. The anal plug became quite prominent and it formed a cone-shaped structure blocking the anal pore by the time the larva was about 60 μ long (Fig. 6). The last few microns of the tail of the microfilaria did not take part in the general thickening of the larval body. Instead the cells, which occupied the tip of the tail, gradually receded into the body of the developing larva with the result that the caudal end of the larva terminated in a small, hyaline but distinct, hooked appendage, composed completely of cuticle (Figs. 6–9). This appendange, being the posterior extremity of the cuticle of the original microfilaria, disappears at the first molt (cf. Figs. 9, 10).

When the larva had grown to a length of about 95 μ and a width of about 25 μ several further changes became evident (Fig. 7). The anterior extremity of the larva ended in a tiny, cone-shaped protuberance which moved in and out and from side to side. The larva occasionally bent its body ventrally. The rectal cells had further increased in size and a lumen appeared in the middle of the coalesced ventral part of the last three rectal cells (*r* 2–4). The cytoplasm of the two cells, which originated from a division of the first rectal cell (*r* 1), now began to merge with that of the other rectal cells. All these cells, especially the *r* 2–4 cells, became exceedingly swollen when the larva was kept in physiological saline. This swelling frequently obscured the smaller cells that originated from the *r* 1 cell. The excretory cell, at this time, increased enormously in size and more granules began to appear in its cytoplasm; the excretory pore began to project slightly above the surface of the body. The cells behind the excretory cell grouped together forming a simple intestine composed of large cells of various shapes and sizes (Fig. 7). This rather cone-shaped group of cells terminated at the rectal cells. Two, or perhaps three, more or less attenuated cells in front of a broad, faintly granular band—the nerve ring—became visible about this time and it is believed that these formed the future esophagus (Fig. 7). The exact origin of these cells was not determined but, as indicated in Fig. 7, they did not appear to grow back from the anterior extremity of the larva. Because of the triradiate structure of the esophagus in the adult, it is likely that three cells,


n
ex p
r1
r2-4
a p
25μ
ex c
3
4
5
6
ip
a pl
7
op
i
8
gp
50μ
9

rather than two, form the esophagus although more than two were never observed at any one time. The larva was exceedingly thick at this stage and the cells that formed the esophagus lay in the center of the body and were surrounded by numerous other cells which tended to obscure them. Nevertheless, the esophagus of *O. fallisensis* appears to originate from cells which lie immediately in front of the nerve ring and in this respect its development is basically similar to that reported in other filarioids (12). The muscle cells enlarged and they formed a pavement of separate, block-like cells with large nuclei beneath the cuticle. The anal plug, flattened out by this time, had a broad convex surface.

When the larva had grown to about 150 μ in length (Fig. 8) it became more active and frequently bent itself ventrally. The rectal cells had further consolidated. The esophagus, well formed by this time, consisted of at least two club-shaped cells the nuclei of which occurred in the thicker ends which touched the anterior cells of the intestine. These esophageal cells tapered gently to the region of the oral opening; they were encircled by the nerve ring about 21 μ from the anterior end. The intestine had further differentiated and it was composed of a double row of globular cells with prominent nuclei; the shapes of the cells gave the border of the intestine an irregular outline.

The larva prepared to molt (Fig. 9) when it was about 210 μ long and had a maximum diameter of 38 μ. The first indication of the impending molt was the loose cuticle which became visible at the extremities as the body of the larva pulled away from it. In this larva the rectal cells had merged to such an extent that their individual outlines were lost, although their nuclei could

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FIGS. 3–9. Developmental stages of *Ornithofilaria fallisensis*.

FIG. 3. Early larva showing slight thickening of body and increase in size of the rectal cells (one rectal cell behind other two), lateral view.

FIG. 4. Early larva showing division of first rectal cell, lateral view.

FIG. 5. Early larva which has thickened and contracted so that the two cells, resulting from the division of the first rectal cell, come to lie immediately above the last three rectal cells, lateral view.

FIG. 6. Early larva which has thickened until it is of nearly uniform diameter from the anterior to the posterior end, lateral view.

FIG. 7. Advanced "sausage-stage" larva with simple intestine. Lumen of rectum forming and cells which will form esophagus visible, lateral view.

FIG. 8. Advanced first-stage larva with well-formed esophagus and intestine, lateral view.

FIG. 9. First-stage larva preparing to molt, lateral view.

NOTE: Key to lettering on Figs. 3–16.

an = anus
ap = anal pore
a pl = anal plug ("nail-like structure" of Brug)
c = cuticle
ex c = excretory cell
ex p = excretory pore
gp = genital primordium
i = intestine
ip = intestinal primordium
lp = lateral protuberance
n = nerve ring
o = esophagus
op = esophageal primordium
p = phasmid
r = rectum
r 1–4 = rectal cells ("genital cells" of most authors)
r 1 = rectal lumen
st = stoma

still be seen; the lumen between them had increased in size. The esophagus was about 43 μ long and was now continuous with the intestine. The intestine, considerably longer than previously, tapered gently backwards and fused with the rectal cells. The genital primordium, about 100 μ from the posterior end, was distinct and consisted of at least two faintly granular cells. The anal plug, the center of which was about 34 μ from the tip of the tail, was broad and flat. The nerve ring and excretory pore were about 27 μ and 36 μ respectively from the anterior end.

When the larva molted, it shed the lining of its esophagus along with the cuticle of the original microfilaria and the second-stage larva was thus attained.

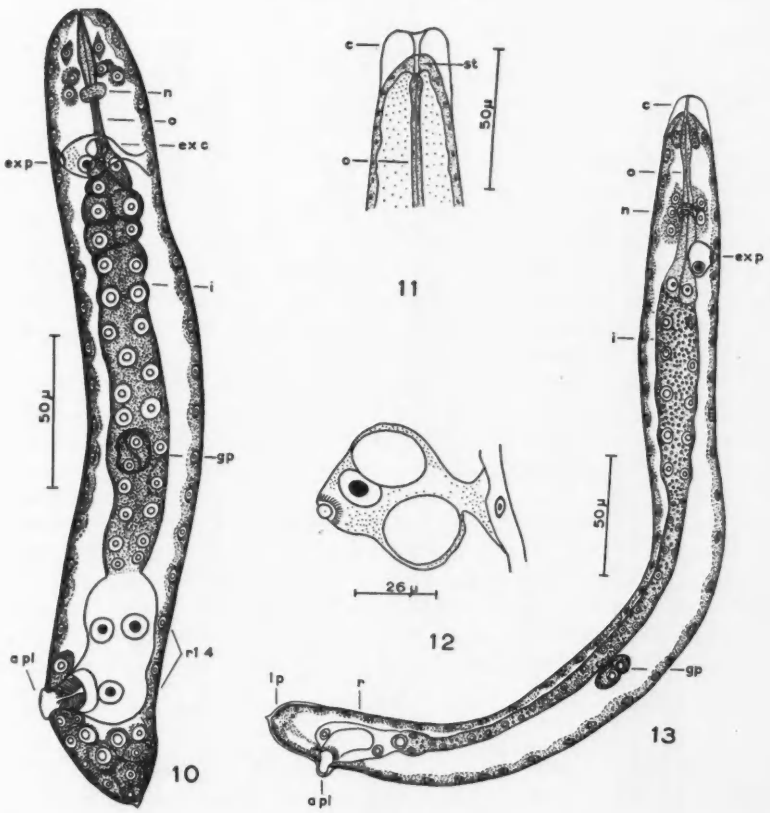
Development to the Second Molt—The Second-stage Larva

A newly emerged, second-stage larva, 250 μ in length and 40 μ in width, had the following appearance (Fig. 10): The esophagus, 57 μ long, terminated anteriorly in a minute buccal capsule which had delicate parallel walls. The nerve ring encircled the esophagus 34 μ from the anterior end. The excretory cell was still large; in some specimens it extended across the body and adhered to the muscle cells on the dorsolateral surface of the body (Figs. 10, 12). The excretory pore was about 54 μ from the anterior end. The intestinal cells were large with prominent round nuclei; the cells of the anterior end still bulged slightly above the surface of the intestine. The rectal cells, which had merged completely, formed a clear mass behind the intestine. The nuclei of the rectal cells were still prominent. The lumen between the rectal cells had enlarged and it terminated in a button-like anal plug 25 μ from the tip of the tail. The genital primordium, consisting of at least two, somewhat attenuated cells, was 111 μ from the tip of the tail. Unidentified cells surrounded the nerve ring and esophagus. The tail of the second-stage larva terminated in a delicate point which was much smaller and more pointed than the prominent, hook-like, terminal appendage of the first-stage larva.

Subsequent growth of the second-stage larva brought an increase in length and a further differentiation of all structures. The esophagus increased in length and the boundary between it and the intestine became obscure. The excretory cell diminished greatly in size. The intestine became narrower, especially posteriorly, and presented a smoother outline. Large refractory granules began to appear in the cytoplasm of the cells comprising the anterior part of the intestine when the larva was about 300 μ long. As the larva grew this granulation spread backwards until, when development in the black fly was completed, the entire intestine had a granular appearance. The lumen of the rectum had greatly increased in size and the syncytium composed of the rectal cells formed the narrow wall of the rectum.

A pair of blunt, lateral protuberances appeared on the caudal extremity when the larva was about 300 μ long; these structures were the harbingers of the second molt. A typical larva (Fig. 13), 310 μ in length, had the following dimensions: maximum width 29 μ; length esophagus 67 μ; nerve ring 36 μ

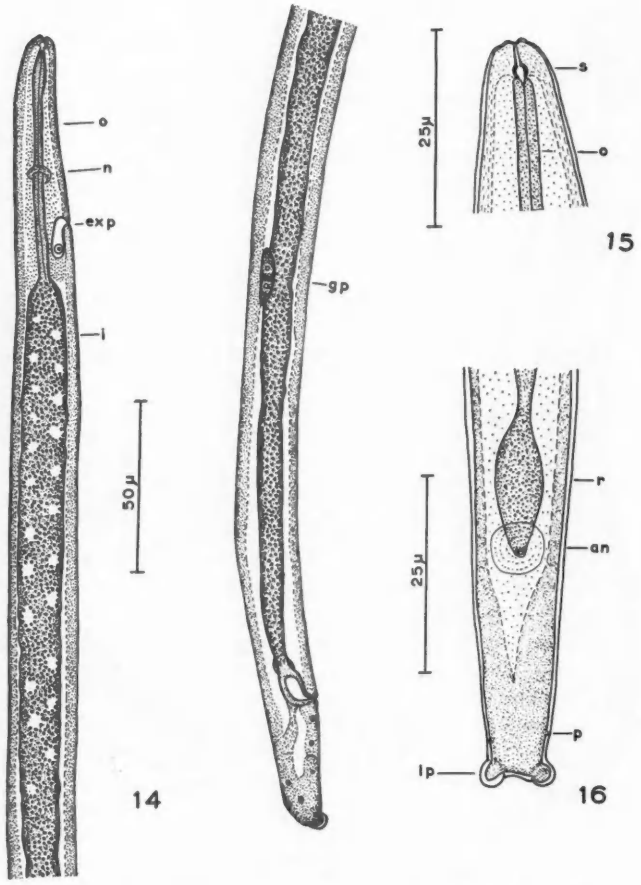
from anterior end; excretory pore 56 μ from anterior end; tail 25 μ in length; genital primordium 128 μ from tip of tail. The cuticle usually became loose at the extremities when the larva was about 300 μ long but the time at which it was shed varied. Typically the second molt occurred when the larva was about 350 μ long but occasionally it did not take place until the larva was as long as 425 μ at which time it was fully grown. The linings of the esophagus (Fig. 11) and rectum were shed during this molt and the anal plug remained attached to the discarded cuticle. After a rapid increase in length, the fully grown, infective, third-stage larva was reached.



FIGS. 10–13. Developmental stages of *Ornithofilaria fallisensis*.
FIG. 10. Newly emerged second-stage larva, lateral view.
FIG. 11. Molting second-stage larva, anterior end.
FIG. 12. Excretory pore and cell in larva illustrated in FIG. 33; an extra vacuole had formed at time it was drawn.
FIG. 13. Second-stage larva preparing to molt, lateral view.

The Third-stage Larva

The dimensions and positions of various structures of 20 third-stage larvae, removed from the heads of black flies, fixed in Looss' fixative, and preserved in glycerine, are as follows: length 389–486 (av. 432) μ; maximum width 18–22 (20) μ; nerve ring 31–58 (49) μ from anterior end; excretory pore 56–86 (65) μ from anterior end; length esophagus 61–97 (77) μ; length tail 27–41 (33) μ; genital primordium 110–151 (133) μ from posterior end. This larva (Fig. 14) is broadest a short distance in front of the middle of the body and it tapers markedly at both extremities. The oral opening is a minute, dorsoventral slit surrounded by a delicate, cuticular border. The amphids



FIGS. 14–16. Developmental stages of *Ornithofilaria fallisensis*.
FIG. 14. Third-stage larva from head of fly, lateral view.
FIG. 15. Anterior end third-stage larva, lateral view.
FIG. 16. Posterior end third-stage larva, ventral view.

occur on either side of the oral opening. Oral papillae were not discerned with certainty. There is a well-defined buccal capsule (Fig. 15). Anteriorly the walls of the buccal capsule are delicate and parallel while deeper they are relatively thick and refractory forming a small subglobular chamber. The esophagus is exceedingly slender—much like that found in the adult worm—and it is surrounded by a mass of faintly granular, dark-staining material which was not identified. The intestine is a thick, tapering tube, the cells of which are packed with granules. The small, elongate rectum leads into a prominent anus which is bordered by a raised, thickened cuticular ring. The nerve ring encircles the middle of the esophagus. The minute excretory pore is associated with a small, elongate excretory cell which extends behind the pore. The genital primordium lies in the posterior half of the worm, apparently connected in some way to the intestine since it moves with the intestine in the body cavity. The tail of the third-stage larva (Fig. 16) is provided with two bulky, rounded, lateral protuberances. A pair of minute phasmids occur about 5–6 μ from the caudal extremity.

No conclusive evidence of a "glandular esophagus" was found in any of the larval stages. In the late first-stage larva the cells composing the anterior part of the intestine were sometimes more spherical in shape than those more posterior and this might give the impression of a "glandular esophagus". Later the cellular structure was almost completely obscured by the numerous granules which appeared in the cytoplasm of the intestinal cells. A slight constriction in the intestine was noted just behind the genital primordium in some third-stage larvae but in all those studied this constriction seemed to be caused by a relative scarcity of granules in the part behind the constriction and not from any fundamental difference in the histology of the two parts. Generally granules occurred in the intestinal cells all the way to the rectum. It is concluded, therefore, that a "glandular esophagus" was not present in the larval stages.

The third-stage larvae migrated from the abdomen to the head when development was completed. In one experiment (No. 11), a number of *Simulium venustum* were allowed to feed on a duck that was heavily infected with *O. fallisensis*. Third-stage larvae were first noted in the heads of these flies eight days later. The remaining flies were subsequently killed 9, 11, and 13 days after they had fed and they were examined for third-stage larvae.

TABLE VI

DISTRIBUTION OF THIRD-STAGE LARVAE OF *O. fallisensis* IN *S. venustum* AT VARIOUS TIMES AFTER INFECTION (EXPT. NO. 11)

Days after feeding	9	11	13
No. flies	12	12	35
Total larvae	38	182	688
Head	34%	53%	71%
Thorax	8%	16%	12%
Abdomen	58%	31%	17%
Av. larvae per fly	3	15	20

The results of these dissections (Table VI) show that, over a period of six days, third-stage larvae originated in the abdomens and migrated to the heads of the flies. Thus, over a period of six days, the number of third-stage larvae per fly increased and the percentage found in the head increased. At 13 days 75% of the infective larvae were located in the heads of the flies and no second-stage larvae were found. The 29% found in the abdomen and thorax at this time was probably the result of movements back and forth from head to abdomen. The infective larvae of *Wuchereria malayi* (Brug, 1927) are reported to move freely from the labium to the abdomen of the mosquito (25). Highby (32) has noted also that the infective larvae of *Dipetalonema arbuta* Highby, 1943 migrate occasionally from the mouth parts to the abdomen of the mosquito.

The third-stage larvae tended to accumulate in the mouth parts of the flies but they were also found in the eyes and about the muscles of the head. When large numbers of larvae were in the mouth parts the slightest pressure often caused them to burst out of the tip of the labella. As many as 48 larvae were found in the head of a single fly, most of them in the proboscis.

Studies on Other Blood-sucking Arthropods as Possible Intermediate Hosts of *O. fallisensis*

Although the foregoing evidence seemed to establish beyond reasonable doubt that various simuliids are the sole vectors of *O. fallisensis* in Algonquin Park, it nevertheless seemed essential to determine whether any other blood-sucking arthropods fed on the ducks, and if so, whether *O. fallisensis* would develop in them. It was important to establish first whether the ducks harbored ectoparasites. Accordingly, careful searches were made for ectoparasites throughout the spring and summer, but none was found (searches for hippoboscid flies at intervals during the day were made after the ducks had been driven slowly into a large flyproof cage). Engorged *Aedes punctor* (Kirby), which presumably had fed on the resting ducks, were captured in a fly trap placed near the duck pens in 1955 by Dr. D. M. Davies. The writer has collected unidentified biting midges (*Culicoides*) after they had fed on the exposed ducks. Therefore, various species of mosquitoes as well as a batch of unidentified biting midges were permitted to feed on a heavily infected duck and were examined later for developmental stages of *O. fallisensis* (Table VII). The results are in contrast to those obtained when simuliids were used and they indicate that mosquitoes and biting midges do not serve as vectors of *O. fallisensis*. *O. fallisensis* will develop to the infective stage in several members of the Simuliidae although some of them like *S. venustum* apparently do not serve as vectors. Similarly if *O. fallisensis* was transmitted by some species of mosquito not tested by the author, one would expect it to develop to the infective stage in others. *Wuchereria bancrofti* is reported to develop in no less than 74 species of mosquitoes of the genera *Culex*, *Aedes*, *Mansonia*, *Anopheles*, and *Psorophora* (24).

TABLE VII

RESULTS OF DISSECTING MOSQUITOES AND BITING MIDGES FED PREVIOUSLY ON A DUCK INFECTED WITH *O. fallisensis*

Species	No. fed	Time dissected (days)	Results
Aedes aegypti	Numerous	Various	Abnormal development in a few
A. communis	24	3–15	One with two "sausage-stages" and one with a first- and a second-stage larva
A. intrudens	16	5–8	One with five "sausage-stages"
A. punctor	9	3–8	Two with "sausage-stages"; one with three second-stages
A. fitchii	4	4–8	All negative
A. stimulans	17	6–7	All negative
Anopheles earlei	15	6–10	Two with three to five "sausage-stages"
Mansonia perturbans	21	7–9	All negative
Culicoides	10	5	Three with "sausage-stages"

OBSERVATIONS ON THE MICROFILAREMIAS OF INFECTED DUCKLINGS

Periodicity of Microfilariae

The microfilariae of several filarioids are reported to fluctuate periodically in the blood of their hosts. Early in the present study it was noted that the microfilariae of *O. fallisensis* fluctuate greatly in the peripheral blood during the course of a single day. To determine if these fluctuations occurred regularly the number of microfilariae in the blood of a duck infected during exposure in 1954 (Duck No. 1481) was estimated from two samples of blood

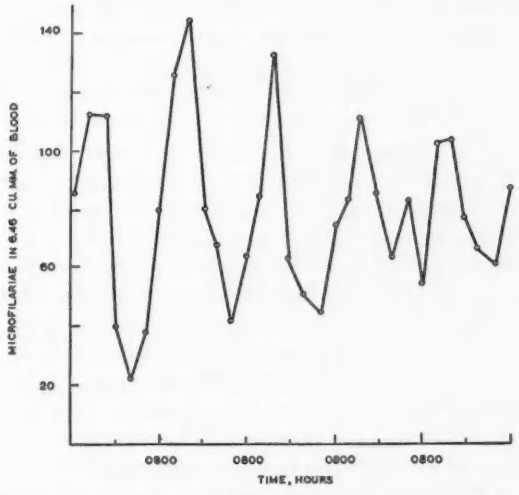


FIG. 17. Changes in the number of microfilariae in the peripheral blood of a duck infected with *Ornithofilaria fallisensis*.

(each 6.46 cu. mm.) taken every four hours from February 1 to February 6, 1955. Fig. 17 shows that microfilariae were most numerous between 1200 and 1600 hr. and were least numerous between 2000 and 0400 hr. The minimum counts were 20–60% of the maximum counts. The differences in the numbers of microfilariae in the samples taken during the day and those taken at night are highly significant ($F = 27$). The differences between the minimum and maximum counts diminished from the first to the fifth day on which the duck was examined; the minimum counts, respectively from the first to the fifth day, were the following percentages of the maximum counts: 20, 29, 34, 50, 60. It is possible that the disturbance of the bird every four hours affected the periodicity of the microfilariae. The vectors of *O. fallisensis* bite almost exclusively during the daytime and their biting more or less coincides with the period in which most microfilariae are present in the blood.

Loa loa (Cobbold, 1864) is apparently the only other filarioid whose microfilariae are reported to be more abundant in the peripheral blood during the day than at night (19, 31). This species, like *O. fallisensis*, is transmitted by a vector which bites during the daytime, i.e. *Chrysops* spp. (15, 39).

Comparison of the Microfilaremias of Infected Ducks

To obtain some indication of the rapidity with which microfilariae accumulate in the blood of naturally and experimentally infected ducks, the microfilaremias of the three ducklings, experimentally infected in 1954, were followed for about 80 days and compared with those of five birds infected during exposure in 1954 (Table I). To minimize the effects of periodic fluctuations of microfilariae, each duckling was examined at a set time each day as follows: No. 1481—1100 hr.; No. 1482—1630 hr.; No. 1484—1500 hr.; No. 1496—1430 hr.; No. 1502—1030 hr.; No. 1503—1330 hr.; No. 1508—1600 hr.; No. 1562—1800 hr. Although the microfilaremias of ducklings infected naturally in 1952 and 1953 were not followed as closely as those in 1954, it is believed, as a result of examining the ducklings at various times, that the picture presented in 1954 was more or less typical of these other years.

The numbers of microfilariae in the blood of the two ducklings 1502 and 1503, inoculated with large numbers of larvae, increased rapidly from the time they were first detected (Fig. 18). This increase was sustained for about two weeks and was followed by a decline and great variability in the number of microfilariae from day to day. After about 40 days the microfilariae increased greatly in duckling 1503 although there was less change in duckling 1502.

The courses of the microfilaremias of ducklings 1502 and 1503 are basically similar to those followed by Webber and Hawking (60) in dogs that were experimentally infected with *Dirofilaria immitis* Leidy, 1856 or *D. repens* Railliet and Henry, 1911. In all these cases there was a sharp and sustained increase in the microfilaremias after microfilariae were first detected, presumably because many of the parasites matured at approximately the same time and there was a more or less rapid influx of microfilariae into the blood. Ducklings 1502 and 1503 were growing, and their blood volume increasing, at

the time they were being examined for microfilariae and this must have tended to depress the concentration of microfilariae in their blood.

The declines in the microfilaremias of ducklings 1502 and 1503 after about two weeks might suggest that the female worms require a period of recuperation after producing a batch of microfilariae. Further studies of experimental infections are necessary, however, before one can profitably discuss this possibility.

The number of microfilariae in the blood of duckling 1562, injected with only 68 larvae, remained low even up to the 80th day. Presumably only a few female worms resulted from the injection of larvae and their microfilarial production was not sufficient to raise the microfilaremia of this bird beyond that indicated in Fig. 18.

The course of the microfilaremia of duckling 1508, infected naturally in 1954 (Fig. 19), is similar to that of duckling 1562, which was injected with only 68 larvae. The former duckling was exposed for three days only and would probably not have received many third-stage larvae during that time.

The course of the microfilaremia of duckling 1496, infected naturally in 1954, was peculiar (Fig. 19). This bird was exposed continuously from June 4

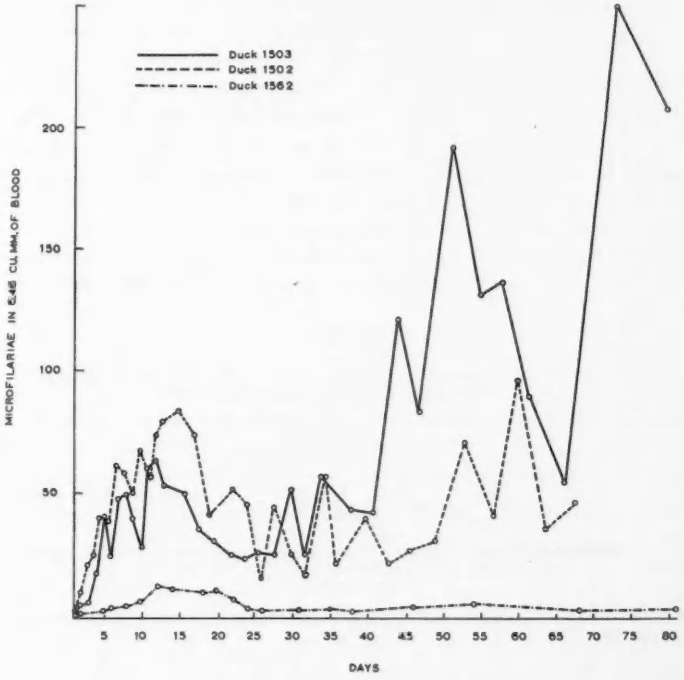
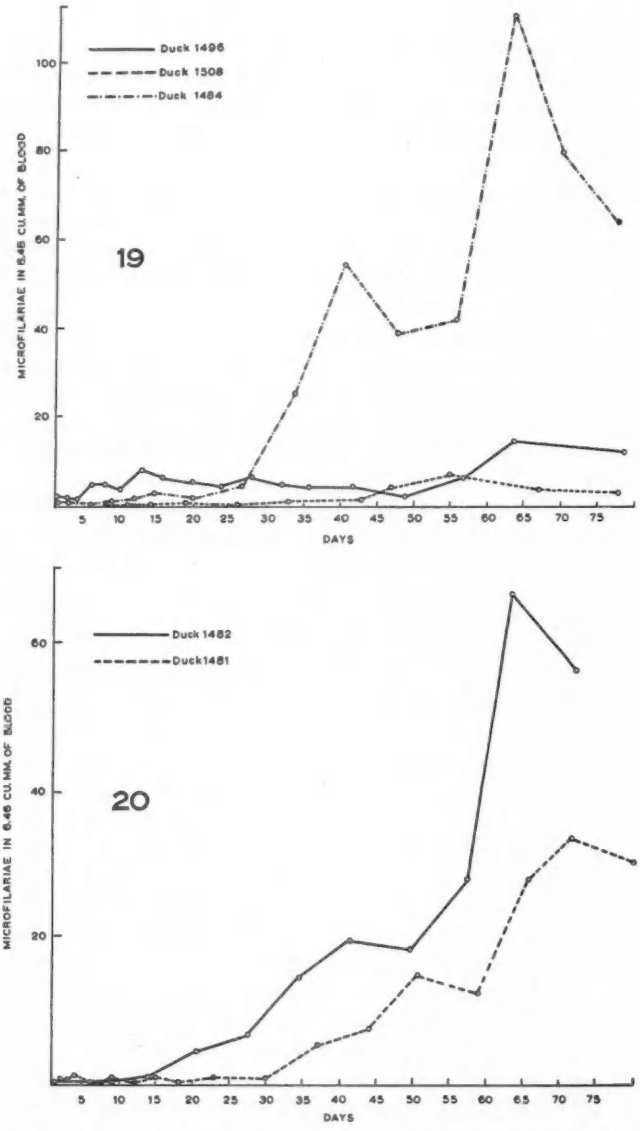


FIG. 18. Microfilaremias of ducklings experimentally infected with *Ornithofilaria fallisensis*.



FIGS. 19 AND 20. Microfilaremias of ducklings infected naturally with *Ornithofilaria fallisensis* in 1954.

(Table I). Nevertheless, its microfilaremia remained low for 80 days although microfilariae were detected in its blood 47 days after it was exposed. Its microfilaremia had increased markedly by the following winter, however (see Table VIII below).

The courses of the microfilaremias of ducklings 1481, 1482, and 1484, naturally infected in 1954, are interesting inasmuch as a period of some three to four weeks, during which few microfilariae were present in the blood, was followed by a period during which the microfilariae increased rapidly (Figs. 19–20). One would have expected a gradual build-up in the microfilaremias rather than the picture presented. The courses of these microfilaremias were different from those found in ducklings 1502 and 1503 injected with large numbers of larvae; in these birds the number of microfilariae increased rapidly in the blood from the time they were first detected. The courses of the microfilaremias were also different from that of duckling 1562, which was injected with only 68 larvae, as well as that of duckling 1508, which was exposed for three days only.

It has already been suggested that ducklings 1481, 1482, and 1484 probably became infected first around June 6. The first microfilariae detected were probably, therefore, the progeny of adults which had infected the ducklings as third-stage larvae about this time. If the ducklings accumulated, through repeated biting of infected flies during the next two or three weeks, only a few more larvae, then one might expect the microfilariae produced by these few worms as they gradually matured to compensate merely for the increasing volume of blood as the ducklings grew. This would cause a lag in the microfilaremias of the infected birds. If, however, an exposed duckling received, later in the fly season, a particularly large number of third-stage larvae in a short period of time, then one would expect a sharp increase in the number of microfilariae as these worms matured at least 30 days later. Figs. 19–20 show that the period of greatest increase in microfilariae in ducklings 1481, 1482, and 1484 occurred about 30 days after they were first detected.

TABLE VIII

MICROFILARIAE IN 6.46 CU. MM. SAMPLES OF BLOOD FROM DUCKLINGS SEVERAL MONTHS AFTER INFECTION*

Date examined	Injected		Exposed				
	1503	1562	1481	1482	1484	1496	1508
Sept. 29	(208)						
Oct. 7–9	212	(3)	(30)	(56)	(64)	(12)	(4)
Dec. 14–16	300	5	43	64	2	17	12
Jan. 31	494		70	73	1	68	5
Mar. 1–2	603	1	100		0	145	9
Mar. 10				330			
Mar. 29	708	5	36	614	2	108	5
Apr. 26	893	1	50	168	2	40	2

**The figures in parentheses are the last figures plotted in Figs. 29, 30 (text).*

It is possible, therefore, that these ducklings received particularly large numbers of infective larvae during the first week in July. It is interesting to note that the heaviest mortality in the ducklings, much of it from *Leucocytozoon simondi*, occurred during the last week in June and the first two weeks in July, 1954, when none of the ducklings exposed for the first time during this period survived. Conceivably *O. fallisensis*, like *L. simondi*, reached its peak of transmission during this period and this might account for the marked increase in the number of microfilariae about 30 days after they were first detected.

The above-mentioned ducklings, examined at frequent intervals for about 80 days, were subsequently examined about once a month to April 26, 1955, about three weeks before the start of the black-fly season in Algonquin Park (Table VIII). The microfilaremia of the duckling injected with 737 infective larvae (1503) increased greatly to April 26 whereas that of the duckling injected with only 68 larvae (1562) remained low. There was considerable variation in the courses of the microfilaremias of birds infected naturally. One (1496) of the two birds with low microfilaremias at about 80 days exhibited a marked increase in microfilariae up to the end of March which was followed by a decrease to April 26, and the other (1508) exhibited no great change. Three other naturally infected birds had relatively high microfilaremias at about 80 days. One of these (1482) exhibited a marked increase up to April 26, another (1481) a slight increase, and the third (1484) a marked decrease. It is difficult, therefore, to predict from observations of the microfilaremias during the first three months whether ducklings will have large numbers of microfilariae in their blood the following spring. Moreover, the microfilaremias of ducks may drop drastically within a few weeks. For example, although 1503 had an estimated 893 microfilariae in 6.46 cu. mm. of peripheral blood on April 26, the number had fallen to 253 by May 19. Similarly ducks 1496 and 1482, which had 40 and 168 microfilariae respectively in 6.46 cu. mm. of peripheral blood on April 26, had far fewer on June 24.

The microfilaremias of ducklings infected naturally in 1952 and 1953 were not followed as closely as those in 1954. Nevertheless, the few observations made on these birds agree with those from the ducks infected during exposure in 1954. Generally speaking, the microfilaremias of these ducks reached their peaks during the late winter or early spring of the year following exposure. After these peaks were reached the microfilaremias declined at varying rates. Of the six birds infected in 1952, two (958, 988) were nearly negative for microfilariae by October, 1953, and two (961, 962) had light infections in October, 1953. Two other birds, however, maintained high, although gradually declining, microfilaremias, into 1954. One of the latter (959) had a high microfilaremia on June 26, 1954, and the other (963) had a high microfilaremia when it died on August 15, 1954. The latter duck was maintained indoors at Toronto after it was exposed in 1952 but the others were re-exposed in 1953 (it will be pointed out later that re-exposure did not alter the courses of the microfilaremias).

Five of the birds infected in 1953 (Table I) were kept for several months after exposure. Two (1111, 1118) were negative for microfilariae by February 1, 1954, and one (1142) had a high microfilaremia on February 1, 1954, but a low one on May 3. The remaining two ducks (1113, 1116) had high microfilaremias at the beginning of the fly season in 1954.

Under certain conditions microfilariae may disappear from the blood of a duck in an unusually short time. One duck (959), originally infected in 1952, maintained a high, although gradually declining, microfilaremia up to the spring of 1954. At this time several successful experiments were conducted using black flies that were fed on this bird. The last time flies were permitted to feed on this bird was June 26. Microfilariae were numerous in the blood at that time. No microfilariae could be found in the blood of this bird on June 30, however, and all subsequent examinations up to July 4, when the bird died, failed to reveal microfilariae. About one week before death this duck stopped eating regularly and became lame. Weakness and emaciation preceded death. The cause of death is unknown. The rapid disappearance of the microfilariae is interesting since injections of microfilariae into clean ducklings have shown that the microfilariae have a life span of at least 30 days (unpublished notes of the author). The rapid loss of microfilariae in this bird may be similar to the "spontaneous cures" reported in humans infected with *Wuchereria bancrofti* (2, 41) and a dog infected with *Dirofilaria immitis* (60). A similar but less spectacular case was that of duck 1491, originally infected during exposure in 1954 (Table I). This bird had many microfilariae in its blood on May 27, 1955, but by June 16 the number had diminished greatly and by June 19 none could be found. No evidence of severe illness preceded the disappearance of the microfilariae in this bird although it was somewhat weak and anaemic at the time the microfilariae disappeared; the subsequent history of this bird will be outlined later.

Evidence of Acquired Immunity

Those ducks which had the highest microfilaremias were usually kept over winter in Toronto and taken to Algonquin Park the following spring to be used in transmission studies. It was thought that the same birds could be used for several years as sources of microfilariae because presumably reinfection each spring and summer would keep the microfilaremias high. Re-exposure, however, was not followed by a rise in the microfilaremias of the ducks. This suggested that infected birds were resistant to reinfection. Observations on some re-exposed ducks from whose blood microfilariae had disappeared are especially significant and these are summarized in Table IX.

Repeated examinations, prior to the black-fly season in Algonquin Park in 1954, failed to reveal microfilariae in the blood of ducks 1111, 1116, and 1118, and re-exposure in 1954 failed to bring about a reappearance of microfilariae in these birds. In contrast, microfilariae appeared in the blood of all ducklings that survived exposure periods starting from May 27 to June 16 (Table I). In 1955 birds were exposed to determine whether this apparent resistance was

TABLE IX

RESULTS OF RE-EXPOSING DUCKS IN ALGONQUIN PARK

No.	Exposed	Results	Negative	First re-exposure	Results	Second re-exposure	Results
1111	May 27/53	+	May/54	May 17/54	−		
1116	June 3/53	+	May/54	July 7/54	−		
1118	June 3/53	+	May/54	May 17/54	−	June 26/55	−
1491	June 4/54	+	June/55	May 3/55	−		
1627	Aug. 12/54	−		June 26/55	+		
1631	Aug. 1/54	−		May 17/55	+		

due to the age of the ducks or to previous infection. Two birds, formerly infected with microfilariae (1118, 1491), were exposed with two adult birds that had never been infected (1627, 1631). Microfilariae did not reappear in ducks 1118 and 1491; these birds were examined repeatedly until October 3, 1955. However, microfilariae appeared in the blood of duck 1627 on August 21 and in the blood of duck 1631 on August 17. It is difficult to believe that ducks 1118 and 1491 were not bitten by flies harboring infective larvae inasmuch as microfilariae appeared in all but three of the 23 ducklings surviving exposure between May 9 and June 26, 1955 (Table II). The data suggest that ducks acquire, as a result of infection, an immunity to *O. fallisensis*.

Litomosoides carinii (Travassos, 1919) is reported to produce immunity in the cotton rat. MacDonald and Scott (40) reported that adult *L. carinii* were shorter and "less mature" in cotton rats that had been infected repeatedly than they were in cotton rats which had not been previously infected.

Unexplained Resistance of Some Ducklings to Infection

In 1955 microfilariae were not detected in the blood of two ducklings although they were examined for several weeks after they were exposed. Duckling 1687 was exposed on June 4 along with five other ducklings (Table II). Microfilariae were detected in the blood of the latter ducklings 36–59 days after they were exposed but no microfilariae were found in the blood of duckling 1687 although it was examined repeatedly for 72 days after it was exposed; this bird died the day following the last examination. Duckling 1741 was exposed on June 26 with five other ducklings. One of the latter (1732) died after it had been exposed for 59 days; no microfilariae were found in its blood. Microfilariae were detected in the blood of all the surviving ducklings 41–51 days after they were exposed except duckling 1741. The latter duckling was examined for 99 days. Duckling 1660 exposed in 1955 (Table II) was also unusual because microfilariae took so long to appear in its blood (88 days after exposure). All these ducklings seemed to exhibit a resistance to infection which cannot be attributed to immunity resulting from previous infection.

Discussion

The present work, demonstrating the transmission of *O. fallisensis* by simuliids, suggests the possibility that simuliids may also transmit other filarioids of birds. Those simuliids that can penetrate feathers seem to be the most likely vectors of filarioids parasitizing nidifugous aquatic birds for these flies are assured of air under the feathers and the flies are in little danger of being wetted or dislodged if the bird submerges itself. Similarly, flies capable of penetrating feathers could be expected to be the most common simuliid pests of many non-aquatic birds which are densely feathered and exceedingly active. Some simuliids, however, which do not penetrate feathers can, nevertheless, feed on certain birds by utilizing unfeathered areas about the head. The young of nidicolous birds might be expected to be more or less defenseless against the attacks of simuliids. The few references in the literature to simuliids attacking birds, in addition to the author's own observations, indicate, as might be expected, that simuliids are pests of a great many birds.

Davies and Peterson (18) report that Dr. C. D. Fowle collected a female *S.* (*E.*) *latipes* in feathers next to the skin of a ruffed grouse. It is probable that other species of simuliids feed on ruffed grouse as well and a knowledge of them would be useful as these birds are frequently infected with filarioids. The author was unable to find larval stages of *Microfilaria fallisi* Brinkmann, 1950 in several species of mosquitoes which had engorged on an infected grouse several days previously. This fact combined with the report of Davies and Peterson suggests the possibility that simuliids may be the vectors of *M. fallisi.*

S. (*S.*) *jenningsi* Mall. feeds around the unfeathered parts of the heads of turkeys in Virginia (35, 56, 57, 58). Similarly *S.* (*S.*) *meridionale* Riley is reported to feed on chickens and turkeys in the southern United States (21, 52).

The studies of Robinson (49) in Georgia indicate that some filarioids of the Corvidae are transmitted generally from adult to nestling birds. There is, unfortunately, little information to indicate which simuliids feed on nestling birds. Bennett (6) has observed engorged simuliids of undetermined species in the nests of crows in Algonquin Park. *S.* (*E.*) *canonicolum* (Dyar and Shannon) was reported by Vargas (59) to feed on nestling owls (reported in Davies and Peterson (18)). The same species also feeds on nestling red-tailed hawks in California and is apparently abundant enough on occasions to cause the death of the young hawks (26). Some simuliids may feed readily on unfeathered nestling birds but not on the fully feathered and active adults. It is difficult to see how such simuliids could be important vectors of filarioids as the infections in young birds must surely originate in the adults. Possibly, however, certain flies, which will not feed on active adult birds, may nevertheless feed on quiescent incubating adults. Filarial transmission would become possible if these flies subsequently fed on the more or less defenseless nestlings.

The status of *S.* (*S.*) *venustum* as a pest of birds needs clarification. This species was collected by Davies and Peterson (18) in swarms over nests

containing unfeathered grackles. The same authors collected three female *S. venustum* as they fed on fledgling crows in Algonquin Park and one as it fed on a sickly great blue heron. The writer collected only three females of this species feeding naturally on domestic ducks in Algonquin Park in 1955 and he has concluded that it is not an important pest of ducks. This species may feed more readily on other birds, however.

This study demonstrates that some caution is required in concluding that a particular experimental intermediate host is a vector of a filarioid in the absence of definite information on its feeding habits. Although *O. fallisensis* will apparently only develop in members of the Simuliidae, it will develop in some of them which, because of their feeding habits, are unlikely to serve as vectors, e.g. *S. venustum* and *S. parnassum*.

The source of the infection of the ducklings which were first exposed (1952) is believed to be wild black ducks (*Anas rubripes*, Brewster) which breed in small numbers throughout Algonquin Park. Microfilarial studies (1) had previously indicated that *O. fallisensis* occurs in black ducks but only recently was the writer able to confirm this by finding a single female *O. fallisensis* under the skin of a black duck captured a few miles from Lake Sasajewan in Algonquin Park. Red-breasted mergansers (*Mergus serrator* Linné) also breed in Algonquin Park but the microfilaria of *O. fallisensis* has not been found in their blood. After 1952, infected white Pekin ducks were maintained in pens near exposed ducklings and these probably acted as the chief reservoirs of infection during the subsequent years of this study. Since microfilariae do not appear in the blood of naturally infected birds until at least 36 days after exposure, it is doubtful if birds infected in any one season contribute much to the spread of the infection until the following spring because by the time microfilariae appear in the blood of the first exposed ducklings the fly season is drawing to a close (cf. Tables II and III). Some time is required, moreover, for the microfilariae to reach appreciable numbers in the blood (Figs. 19–20). Therefore the sole reservoirs of infection, during most of the black-fly season in any one year, are probably old birds which were infected in previous years.

One would expect the period of transmission of *O. fallisensis* to be similar to that of *Leucocytozoon simondi* since they are transmitted by the same vectors. Generally, however, the transmission of *O. fallisensis* ceases several days before that of *L. simondi* (Table I). In 1955, ducklings exposed up to July 25 became infected with *L. simondi* although the last birds to become infected with *O. fallisensis* were exposed on July 2. After July 1, *S. rugglesi* was scarce; only 13 were caught in six evenings of fly trapping (Table III). This suggests that the number of flies necessary for the transmission of *L. simondi* is much lower than that necessary for the transmission of *O. fallisensis*. In a wild population of flies the number infected with *L. simondi* could be expected to be greater than those infected with *O. fallisensis*. Several factors would cause this: e.g. (1) the probability of a fly ingesting *L. simondi* is undoubtedly greater than the probability of its ingesting microfilariae because the parasites of the former are usually much more abundant than the

latter in the blood; and (2) the development of *L. simondi* to the infective stage takes less time than that of *O. fallisensis* (23). If the number of flies infected with *L. simondi* is greater than the number of those infected with larval *O. fallisensis*, a much smaller fly population would be necessary for the transmission of the former. This would explain why the transmission period of *L. simondi* is longer than that of *O. fallisensis*. It would also explain why birds exposed from May 12 to June 7 in 1953 (Table I) did not become infected with *O. fallisensis* although they became infected with *L. simondi*. The transmission of *Onchocerca volvulus* (Leuckart, 1893) to humans in Central America is believed to depend not only on the presence of the proper simuliid vectors and infected persons but also upon their respective population densities (27).

Within the genus *Onchocerca* are found the only species other than *O. fallisensis* which are known to utilize members of the dipteran family Simuliidae as intermediate hosts. *O. gutturosa* of cattle is transmitted by *Simulium ornatum* (54) and *O. volvulus* of humans by *S. damnosum* in Africa (7, 8), and *S. metallicum*, *S. ochraceum*, and *S. callidum* in Central America (28). *O. reticulata* and *O. gibsoni*, of horses and cattle respectively, are transmitted, on the other hand, by various species of biting midges (*Culicoides*) (10, 53). Species of *Onchocerca* differ from *O. fallisensis* and other filarioids whose life cycles have been studied, however, in that the microfilariae reside in the skin and rarely if ever are found in the circulating blood. The microfilariae are ingested, presumably in tissue fluids, as the fly is feeding.

The development of the microfilaria of *O. fallisensis* in the black fly is similar to the development of other dipetalonematids in their intermediate hosts. Fig. 1, describing the changes which take place in the dimensions of the developing larvae of *Ornithofilaria fallisensis*, is similar to one given by Feng (25) for *Wuchereria bancrofti* and one given by Kartman (37) for *Dirofilaria immitis*. The changes in the dimensions of other species have not been followed closely but it can be inferred from illustrations and descriptions of the larval stages given in the literature that most develop similarly. The peculiarity of the growth of the larvae of these species is the formation of a "sausage-stage"—a form which has thickened and contracted until it is shorter and several times thicker than the original microfilaria. However, *Dipetalonema blanci* and perhaps *Conispiculum flavescens* are reported to develop from the microfilaria to the infective stage without the intervention of a "sausage-stage" (4, 11, 43, 47).

The finer details of the morphological changes which take place during the development of the microfilaria to the infective stage have been determined with some degree of completeness in *Wuchereria malayi* and *W. bancrofti*. Feng (25), continuing studies initiated by earlier workers, described in detail the development of *W. malayi* in the thoracic muscles of *Anopheles hyrcanus*. Chandler *et al.* (12) have given an excellent series of illustrations showing the development of *W. bancrofti;* unfortunately no text accompanies these illustrations.

Larvae of *O. fallisensis* lack a posterior part to the esophagus generally referred to as the "glandular esophagus". Larvae of *W. bancrofti* and *W. malayi*, on the other hand, possess a "glandular esophagus" derived from cells similar to those that form the anterior part of the intestine in *O. fallisensis*. The anterior part of the esophagus—the so-called "muscular esophagus"—is apparently formed in *W. bancrofti* from two or three elongate cells which appear in front of the nerve ring in the early first-stage larva. Later these cells are joined posteriorly by the column of cells that ultimately form the "glandular esophagus". This development is similar to that in *O. fallisensis* except that (*a*) the cells that form the "muscular esophagus" of *O. fallisensis* extend behind the nerve ring and (*b*) the cells that join them posteriorly ultimately form the anterior portion of the intestine rather than a "glandular esophagus". According to the figures given by Feng, the anterior part of the esophagus of *W. malayi* is composed of several cells that are early associated with more posterior cells, the latter forming the glandular portion of the esophagus.

Formation of the rectum from the rectal cells (formerly regarded as genital cells) agrees with the findings of Yamada (62), Feng (25), and Chandler *et al.* (12). During the development of *W. malayi* the first rectal cell is reported to divide at 24 hr. and each daughter cell at 34 hr. Each of the last three rectal cells also divides at 34 hr. with the result that 10 cells form the rectum of the third-stage larva. The rectum of *O. fallisensis*, however, is formed from only five cells, the three last rectal cells and two cells originating from a division of the first rectal cell. This is similar to the development of the rectum in *W. bancrofti* as illustrated by Chandler *et al.*

The prepatent period of *O. fallisensis* in experimentally infected birds is 30–36 days. Little is known of the prepatent periods of other filarioid worms because there have been few attempts to transmit them experimentally or to collect epizoological information which might provide an estimate. *Dirofilaria immitis*, the first filarioid to be experimentally transmitted, is said to have a prepatent period of some eight to nine months (5, 60). Highby (33) allowed mosquitoes infected with larvae of *Dirofilaria scapiceps* to feed on rabbits and he found microfilariae in the blood of one 286 days after it was bitten and in another 391 days after it was bitten. These were undoubtedly exceedingly light infections as he failed to recover adult worms. The prepatent period is probably shorter under natural conditions. The prepatent period of *Conispiculum flavescens* is estimated to be about 69–72 days (43, 47). Chardome and Peel (13) have estimated that the prepatent period of *Dipetalonema streptocerca* is about three to four months and *Dipetalonema perstans* about 9–12 months. They also report microfilariae of *Loa loa* in the blood of a child one year old. Jordan (36) has reported microfilariae of *Wuchereria bancrofti* in two children less than one year old. The prepatent period of *Litomosoides carinii* of the cotton rat is from 50–70 days (38, 61). The interesting report of Zekhnov (63), who found microfilariae in the blood of nestling ravens that were 9–16 days old, indicates that some of the avian filarioids have even shorter prepatent periods than *O. fallisensis*. Robinson

(49) has estimated that filarioids of unknown species in jays and crows mature in about eight weeks. Chernin (14) has reported the prepatent period of an unknown filarioid in domestic ducks exposed in Michigan as six to nine months. This estimate was based, however, on microfilariae found on blood smears and it is highly probable that he failed to detect microfilariae until long after they appeared in the blood.

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